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THE PRINCIPLES

OF

PATHOLOGICAL HISTOLOGY

BY

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WITH AN INTRODUCTORY NOTE

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TO

PROFESSOR JOHANNES ORTH,

GEHEIMER MEDIZINALRAT,

DIRECTOR OF THE PATHOLOGICAL INSTITUTE OF THE
UNIVERSITY OF GÖTTINGEN,

THIS BOOK IS RESPECTFULLY DEDICATED

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PREFACE.

THIS work is intended to supplement the regular courses in Pathological Histology and to lead the student and graduate to the application of such knowledge in practice.

The subject is one in which adequate illustrations are peculiarly necessary. In laying the ground work, diagrams are more useful for the purpose of emphasizing the elementary forms with which the student must first become familiar. In practical life, however, he must be qualified to interpret the appearance of actual objects seen through the microscope. A book to confer this ability must present the histology of the various diseases as faithfully as the microscope itself.

Accordingly no effort or expense has been spared in illustrating the present volume. Special apparatus was devised for the optical and photomicrographic work, and the negatives thus secured have been reproduced by the Heliotype process, selected as the best after careful trial. The authors desire to acknowledge the invaluable assistance and coöperation of R. Winkel, of Göttingen, and Voigtländer & Son, of Braunschweig, by whom the improved optical apparatus was made, and to compliment the Heliotype Company, of Boston, for their faithful reproductions.

In Part I. will be found the most trustworthy methods for preparing tissues for microscopic examination. It is so arranged as to be equally useful for the student and for the practitioner. Attention may be called to the simplicity of the present freezing microtome technique as contrasted with the difficulties of the paraffine or celloidine methods.

Part II. includes the principal portion of the work and deals with the pathoogical histology of organs.

Part III. contains a simple exposition of the optical problems with which the student should be familiar, and more technical directions for those who wish to take up the subject of photomicrography.

For the guidance of the student, but without any attempt at completeness, we have placed at the foot of the pages references to the literature bearing on the subjects under consideration.

The chapter on the Blood has been treated from a practical and clinical standpoint. For the text of this chapter and the colored plate we are indebted to Dr. Irving Phillips Lyon, of Buffalo.

To Professor Orth we are under obligation for placing at our disposal the apparatus and material of the Göttingen Institute, and to the following gentlemen for preparations or material, namely: Professor Marchand, Professor Councilman, Professor E. Kallius, Professor Williams, and Professor Spiller, to

Prosectors Georg Schmorl, E. Benda, and P. Guipel, and to Drs. Futcher, Kirkbride and Betts.

In conclusion we desire to express our appreciation of the kind interest and encouragement of Professor William H. Welch, of Johns Hopkins University, to whom the following Introduction is due.

HARVEY R. GAYLORD, M.D., LUDWIG ASCHOFF, M.D.

BUFFALO, October, 1901.

INTRODUCTION.

Both teachers and students will welcome the publication of Drs. Gaylord and Aschoff's "Principles of Pathological Histology."

This work is distinguished from other text-books on morbid histology of similar scope especially by the extensive use which has been made of photomicrographs to illustrate the lesions of disease. It is not necessary to discuss here the respective merits of drawings and of photographs for such illustrations. Each method has its special advantages and fields of application. When such excellent results of the photographer's art are presented as will be found in the plates of this book, there can be no question of the material aid which they afford to the student, especially to one who has acquainted himself with the actual appearances of the corresponding lesions under the microscope. The amplifications have been judiciously chosen, and the subjects selected for illustration are those which are usually studied in practical laboratory courses in pathological histology. These features adapt the work especially for the use of students working in the pathological laboratory.

The subject-matter of the text has an especial value as the presentation of the experience of the authors based upon the methods and teachings of Professor Orth's excellent laboratory in Göttingen. The section on the technique of photomicrography cannot fail to interest and aid practical workers in this important field.

William H. Welch.

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PATHOLOGICAL HISTOLOGY.

PART I.

MICROSCOPICAL TECHNIQUE.

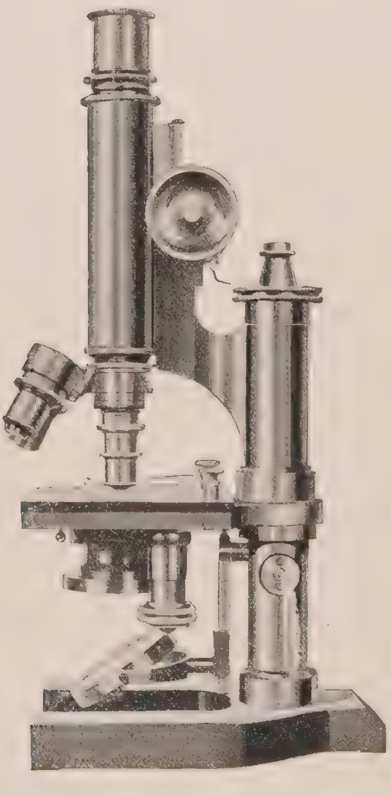
GENERAL REMARKS.

THE optical portions of the student's microscope must be kept scrupulously clean. The slightest deposit upon the front lens of an objective is sufficient to seriously disturb the correction of the entire system. Canada balsam, water or glycerine, the reagents most commonly used in embedding preparations, are the greatest offenders in this connection and the student should establish a habit of occasionally examining and carefully cleaning the fronts of his different objective systems. If Canada balsam is deposited upon the front lens, this must be carefully removed with a soft piece of linen moistened in xylol or turpentine. Alcohol should never be used for this purpose as the front lenses of most objective systems are cemented in place with shellac, which is readily dissolved in alcohol, but is not affected by xylol or turpentine. The immersion oil should be carefully removed from the oil immersion lens directly after use, and never allowed to dry upon the mount. A soft piece of old linen or specially prepared Japanese tissue paper should be used for this purpose. The mirror of the microscope must be occasionally dusted, and both ends of the ocular treated in like manner. A soft camel's hair brush, or one improvised by wrapping a piece of frayed linen about a small stick, should be used.

Having determined that the instrument is in suitable condition it is placed before a window, preferably one facing to the north, and the mirror is so arranged that light—when possible that reflected from clouds—is projected into the axis of the microscope tube. A simple method for determining whether the light is properly reflected, is to remove the ocular, when a bright image of the window may be seen projected by the upper lens of the objective. By moving the mirror this image is readily brought to the center, when the ocular may be returned. The illumination will then be sufficiently central for the student to proceed. The tube of the microscope is now raised by means of the rack and pinion. A slide is placed upon the stage and the tube is gradually lowered until an image of the object is seen in the ocular. The illumination is readily controlled by means of the iris diaphragm, with which every modern instrument is supplied. For observation with low power many instruments are fitted with an iris diaphragm in the plane of the stage, to be used when the condenser is removed. The condenser is only intended for observation with high powers. With objectives up to eight millimeters, the condenser should be removed and the illumination controlled either with a cylinder diaphragm or with the diaphragm in the plane of the stage. It is occasionally convenient to raise and lower this small iris diaphragm, for which purpose it should be connected with the rack and pinion of the substage and should not be permanently fixed in the plane of the microscope stage. The concave mirror serves to increase the amount of light when the condenser is not used. The condenser should only be used in conjunction with the plane side of the mirror.

In using the oil immersion the drop of oil should be placed upon the front of the objective with a small brush or wire loop when the objective should be carefully lowered by means of the rack and pinion, until the drop of oil comes in contact with the cover-glass of the preparation, after which the eye must be returned to the ocular, and the tube slowly and gradually lowered, taking great care that the focal point is not passed and that the objective is not pressed upon the preparation. Accidents of this sort are exceedingly common with beginners and should be carefully avoided. To make sure that the objective has not come in contact with the preparation, the latter should be moved slightly from side to side while the objective is being lowered. By this means any pressure upon the surface of the preparation may be readily detected. All instruments used in mounting preparations, in particular, needles, slides and cover-slips, must be kept free from dirt. The introduction of foreign particles, such as wool, silk, particles of dust, etc., commonly leads the beginner to erroneous conclusions.

FIG. 1.



Student's microscope with simple substage, small iris diaphragm in the plane of the stage, and revolving nose piece fitted with 2 mm. ($\frac{1}{12}$) oil immersion, 4 mm. ($\frac{1}{6}$) and 16 mm. ($\frac{2}{3}$) dry objectives.

Slides and cover-slips are first washed in water or weak alcohol and then placed in a suitable wide-mouthed bottle containing used absolute alcohol. Waste alcohol should be preserved in a large-mouthed bottle and into this may be placed soiled cover-slips and slides, from which the balsam or other gummy materials will be readily dissolved, after which they may be easily cleaned. Slides to which paraffine sections are to be attached must be thoroughly scrubbed with warm water and soap and then thoroughly cleaned in alcohol. A ready method for determining whether all fatty materials have been removed from slides consists in pouring a few drops of water upon their surfaces. If the slide has been thoroughly cleaned the water will spread out upon the glass, otherwise, it will be retracted into drops, indicating that the surface is coated with some greasy material.

The examination of all microscopic preparations should begin with the lower powers. Only when the observer has ascertained the various structures of which the preparation is composed, and, as far as possible, their relations to each other, should a particular portion of the preparation be observed with high magnification. The student must learn to recognize by low magnification, normal structures in the preparation, such as glands, muscle fibers, etc. The illumination must be properly adjusted to suit the character of the object under examination. The delicate outlines of unstained cellular elements as they appear in fluids and excretions, can only be recognized when faintly illuminated. Under brilliant illumination they commonly become invisible, and for this reason all fresh preparations should be examined with the iris diaphragm well contracted. Stained preparations, especially when observed with high powers, require brilliant illumination, and should be observed with the full opening of the condenser. Increasing the magnification of an objective by using a high ocular, is a doubtful expedient. The ocular serves only to magnify the image produced by the objective, and never increases the resolving power of the system. The high magnification of the ocular is associated with a great loss of light, aside from which the short focal length of the upper lens requires a distinct effort at accommodation on the part of the eye, which is very fatiguing if long continued.

A. METHODS FOR THE MANIPULATION OF FRESH MATERIAL.

The investigation of pathological material should, whenever possible, begin with an examination of the material in the fresh state. In many cases this method is an absolute necessity, as many changes, *i. e.*, fatty infiltrations, glycogen, and uric acid deposits are removed by the chemical reagents used in hardening or embedding the tissue. Glycogen and uric acid are dissolved in dilute alcohol, fat in absolute alcohol. Many fine changes in the protoplasm, such as albuminous granules in cloudy swelling, become invisible when the tissue is embedded in highly refractive material, such as Canada balsam. The fresh method is applicable to the investigation of both fluids and fresh tissue. Where a fluid is to be investigated a drop is placed upon a slide by means of a pipette and covered with a cover-glass, which should be dropped slantingly upon the surface of the fluid, to prevent the accumulation of air bubbles beneath it. An excess of fluid is removed by blotting the surface of the slide with a piece of filter paper. The ideal amount of fluid is exactly sufficient to fill the capillary space between the cover-glass and slide. A thick fluid, or one containing a large number of cellular elements, should be diluted with normal salt solution (0.75 per cent.). A ready method is to place a drop of salt solution upon the slide and introduce into it a small amount of the fluid to be examined. When searching for cellular elements in a large amount of fluid, the entire specimen should be placed in a conical glass until sedimentation occurs, or, where time is a factor, or the convenience at hand, a centrifuge will accomplish the same result in a much shorter space of time.

There are several methods of examining fresh material, each of which has a special application. The slide upon which the fresh material is to be examined should first be prepared by placing upon it a drop of an indifferent medium, into which the fresh tissue is introduced. This prevents the preparation from drying, which results in distortion and alteration of the proportions of the tissue.

I. Examination of Surface Scrapings.

The material in this method is obtained by passing a knife blade lightly over the surface of a membrane or the cut surface of an organ, and examining in normal salt solution the fluid which flows upon the surface of the knife. Where the fluid thus obtained is thin, the salt solution may be dispensed with and the material examined directly after being placed upon a slide and covered with a cover-glass in the usual manner.

II. Examination of Teased Particles.

The denser structures of the human body may not readily be examined under the microscope until broken up into fine particles. These particles should, as nearly as possible, represent the elements of which the tissue is composed. The looser structures may be readily detached with a needle, but the particles of the denser tissues are best obtained with a curved shears, by cutting a small particle from the surface of the organ. The beginner invariably takes too much material, but a few attempts will show him that an exceedingly small particle of tissue is sufficient. The scrap of material is placed in a drop of salt solution upon the slide and teased with a pair of needles until all is broken up into minute particles. During this manipulation the slide should rest upon a black background, in order that the student may more readily see the light-colored particles of tissue. When the greater portion of the tissue has been broken up, the larger pieces are removed and the remaining fragments are covered with a cover-glass. Occasionally, where the tissue to be examined is very dense, it is necessary to soften it by placing it for a period of time in a so-called macerating fluid. The period of time required to macerate small particles of tissue is one to two days, and the solutions used for this purpose are the following:

1. Thirty-per-cent. alcohol used for the isolation of epithelial elements. (So-called Ranvier's $\frac{1}{3}$ alcohol.)
2. Thirty-three-per-cent. potassium hydrate solution (freshly prepared); especially useful in isolating muscle fibers. The examination of the material macerated in this fluid must be made in a drop of the fluid itself. Dilution of the fluid causes shrinking and distortion of the material, as the result of osmosis between the fluid and the dilutant.
3. Pure sulphuric acid, used in the isolation of glandular structures, such as uriniferous tubules in the kidney. The tissue must be thoroughly washed in running water before examination.
4. Müller's fluid (potassium bichromate 2.5; sodium sulphate 1; aqua dest. 100). Müller's fluid is especially useful in the isolation of nerve elements.

The dish containing the macerating fluid and the material undergoing maceration must be covered to prevent concentration of the solution by evaporation.

III. Examination of Fresh Material by Mashing.

Many excretions and crystalline deposits are best examined in the fresh state by crushing the material between a slide and cover-slip. This method has likewise a wide range of applicability in the examination of pathological nerve structures. A small portion of the material is placed upon a slide, covered with a cover-glass and exposed to pressure until the material spreads out between the slide and slip. The addition of fluid is not desirable, and should be omitted.

IV. Examination of Fresh Tissue by Section.

For purposes of orientation, thin sections of the tissue, even in the fresh state, are indispensable. These may be prepared in various ways, of which the following are the most useful:

1. **With the Section Razor.**—The ordinary razor, if sufficiently sharp may be used for this purpose. To prevent the section adhering to the razor, it is advisable to moisten the blade in a dish of water or salt solution before each section is cut. During the process of cutting, the tissue is held firmly between the thumb and index finger of the left hand. The blade of the knife is drawn

from the heel to the point over the surface of the material, endeavoring to cut from the surface of the tissue as thin a section as possible. In making the cut the arm and hand are held rigid and the razor is drawn rapidly through the tissue by a motion confined to the shoulder joint. The blade should retain its original angle in relation to the tissue during the entire movement. The thin slice of tissue is transferred directly from the blade of the razor to a slide prepared with a drop of fluid, and is best manipulated with a camel's hair brush moistened in salt solution. When the section has reached the slide it is carefully spread out and covered with a cover-glass in the usual manner. Soft tissues are not readily or satisfactorily cut by this method, and for such material the section razor is properly displaced by the next method.

2. **With the Double-bladed Knife.**—This instrument consists of two thin blades placed parallel to each other, so mounted that the distance between the blades may be adjusted. For soft tissues the distance between the blades must be greater than where hard tissues are cut, and the exact distance required must be ascertained by trial. Before each attempt the knife must be immersed in a dish of water, in order that the space between the blades may be filled with fluid. The section is made perpendicular to the surface of the organ or tissue, and is finished by turning the knife in its long axis and making a short horizontal movement at right angles to insure the separation of the section at its base. The knife is again returned to the dish of water and the blades opened, when, if the attempt has been successful, the thin section of tissue will float out upon the surface. The successfully obtained section is drawn upon the section lifter and transferred to a wet slide. After being carefully spread out the excess of fluid is drained from the slide and the section covered with a cover-glass.

3. **With the Freezing Microtome.**—Fresh tissues may be readily cut with a freezing microtome, the liver and kidney in particular being well suited to this method. (For technique of the freezing microtome see page 29.) Sections when prepared are placed in a dish of previously boiled water, in which any air bubbles adhering to the preparation are promptly absorbed, after which the section is spread out and covered upon a slide, in the manner already described for the preceding methods.

V. Reagents.

While the simple fresh preparation permits an examination of the size and form of the cells, little or nothing may be learned of the character of their nuclei, as the protoplasm of the cell body in the fresh state possesses the same or nearly the same refractive index as the nucleus, which prevents the latter from appearing as a distinct structure embedded in the cell. The examination of fresh tissue in salt solution or water, is of equally little value for the demonstration of foreign substances in the cell body, such as fat, pigment, albuminous granules, glycogen, etc., for the reason that the exact location in the tissue of these materials must be accurately determined. To render the tissue transparent and to bring out the differences of refractive index between the different constituents of the cell, certain chemical solutions are profitably employed. These solutions are known as reagents. In order that the reaction of the tissue to the chemical reagent may be observed under the microscope, the solution must be gradually brought in contact with the preparation. By this means the tissue which has been exposed to the action of the reagent may be compared with the untreated portion of the specimen, and the gradual appearance of the reaction may be observed while a mental picture of the tissue in the fresh state is retained. A ready method of applying the reagent to the tissue consists in applying a drop of the reagent to the edge of the cover-glass, which has previously been placed upon the section, and aiding the diffusion of the reagent through the preparation by applying a small piece of filter paper to the opposite edge of the cover-slip. During the process of diffusion the tissue is observed through the microscope. The reagents commonly employed and which should be found on the desk of every student of pathology are:

1. **Acetic Acid (2.5 per cent.)**.—This reagent causes the protoplasm of the cells to become transparent without altering the nuclei, for which reason the latter appear sharply defined within the cell body. Besides nuclear structures, fat droplets, elastic fibers and bacteria remain unchanged and become sharply defined against the more or less transparent protoplasmic structures. Acetic acid causes stringy coagulation of mucus.

2. **Potassium Hydrate (2.0 per cent.)**.—This causes clearing of the protoplasm, and nuclei of the cells, but does not alter elastic fibers, fat, bacteria and certain kinds of pigment.

The indications for the use of either of these reagents are as follows: In examining a section of fresh tissue to determine the size, character, relation and position of the nuclei, the preparation is treated with acetic acid. As the reaction of the protoplasm to the acetic acid develops slowly, the boundaries of the cells remain distinct for a sufficient period of time to fulfill all requirements of orientation. Where the principal object of the examination is to determine the relation, distribution and amount of fat in the cells, potassium hydrate should be used. The application of this reagent to the tissue is followed by a clearing of the entire structure, leaving the fat droplets sharply defined in the form of spherical, usually colorless spherules of varying size. Bacteria which may be present are distinguished from the fat droplets either by their form (bacilli) or when cocci by their uniformity in size and arrangement in groups or chains. Pigment may usually be distinguished from either of the above by its color and a further distinction is usually found in the angular form of the granules. A difficulty in distinguishing between fat and pigment can only arise where the fat possesses a distinct yellow coloration and the pigment occurs in spherical granules, in which case the differentiation depends upon the relation of the bodies in question to stains, hardening solutions, and solvents.

The greater part of human fat is readily stained with osmic acid, with which it forms a chemical combination and takes on an intense black. Two of the aniline dyes, Alkanna and Sudan III. stain it a deep red color.

Bacteria and most pigments are not affected by either of these stains.

Fat is readily soluble in ether; bacteria and pigment remain unchanged.

Pigment when formed in the tissue is readily disintegrated by concentrated sulphuric acid.

Bacteria are readily stained with suitable aniline dyes (fuchsin, Bismarck brown, etc.), which do not color either fat or pigment.

Elastic fibers which remain unchanged in tissues treated with potassium hydrate may be readily differentiated from pigment by their characteristic spiral form and definite contour.

3. **Hydrochloric Acid (5 per cent.)**.—Salts of lime are commonly deposited in the tissue under pathological conditions. A similar process occurs normally in the calcification of bone in young persons. The pathological deposits consist of fine granules of carbonate or phosphate of lime. Both of these salts may be readily dissolved with dilute hydrochloric acid. Carbonate of lime, when treated with this reagent, liberates carbonic acid gas. The reaction may be observed under the microscope and the gas may be seen in the process of formation issuing in small bubbles into the fluid in which the specimen is examined. If sul-

phuric acid be added to fresh preparations containing lime deposits, needle-shaped crystals of sulphate of lime are formed.

4. Iodine Solution. (**Dilute Lugol's Solution**):

Iodine pur.,	1.0
Potassium iodide,	2.0
Aqua dest.,	300.0

This reagent is employed to detect certain substances with which it gives characteristic color reactions. These are glycogen which it stains a deep brown and which appears in the form of large drops, distending the protoplasm of the cells in which it is deposited; starch, with which it gives a blue reaction, and amyloid, which it stains brown, similar to glycogen, from which it may be differentiated by its high refractive index and distribution in the tissue. It is occasionally desirable to preserve, for purposes of demonstration, specimens of fresh tissue which have been treated with iodine. This may be accomplished by smearing the edges of the cover-slip with vaseline, or sealing them with melted wax or paraffine. Where the preparation is to be kept but a short time it may be laid in a so-called "moist chamber," which is nothing more than a covered dish, in which a few pieces of moistened filter paper have been laid. A convenient support for the preparation may be made of wire.

5. Silver Solution. (**1-per-cent. Aqueous Solution of Silver Nitrate.**)—This reagent is used for the demonstration of the cementing substance which unites the opposing edges of the single layer of epithelium which lines the serous cavities, lymph spaces, and blood vessels. The reagent is applied by placing small pieces of detached epithelial membrane for a period of one to two minutes in a 1-per-cent. aqueous solution of silver nitrate, at the end of which time the tissue takes on a cloudy white appearance. After removing from the silver solution, the membrane is carefully rinsed in water and spread out upon a slide, embedded in glycerine, covered with a cover-glass, and exposed for a short period of time to the action of sunlight. The appearance of the reaction between the silver and the cement substance may be observed under the microscope, and takes the form of a gradual blackening, which increases until the lines of union and the interstices between the cells are sharply defined, an intense black network enclosing the unstained epithelial elements.

The nuclei of the cells may be stained with carmine, to accomplish which the membrane must be thoroughly washed in distilled water, to which has been added a few drops of glacial acetic acid, then placed in the carmine solution, and after staining, embedded in glycerine.

B. METHODS FOR THE PREPARATION OF HARDENED MATERIAL.

I. Fixation and Hardening.

The natural limitations of the fresh method of examination are such that it will seldom suffice for the complete solution of a pathological problem. Any further step necessitates fixation and hardening of the tissue before the more elaborate procedures of embedding, staining, etc., can be carried out. The fixation of the tissue must precede the hardening process. Most solutions used for fixation have a hardening effect upon the tissue, but where this is not the case and the solution used simply fixes the tissue, hardening must be accomplished by the use of a separate hardening agent.

It is well to note that no fixing and hardening solution produces equally good results with different kinds of tissue, and that there is no one solution which, in every case, gives the best results.[1] For this reason the character of the investigation to be undertaken and the special constituents of the tissue which are to be examined will indicate which fixing solution or solutions are to be employed.

Pieces of tissue intended for fixation and hardening must not be larger than one-half to one cubic centimeter, in order that the fixing solution may penetrate the tissue rapidly and evenly. For the same reason the fixing and hardening solution must be of liberal quantity (never less than ten times the volume of the block of tissue), and must come in contact with all sides of the specimen, which should be supported by covering the bottom of the bottle with small wads of paper or cotton batting.

After fixation the preparation is seldom sufficiently hardened for sectioning, and the treatment with hardening agents must be followed by a second treatment which consists either in freezing the tissue or infiltrating it with a substance which will give it the necessary rigidity. The latter method is known as embedding, and is preceded by thoroughly washing the tissue in running water twelve to twenty-four hours and dehydrating in alcohol. The treatment with alcohol serves as a form of second hardening and consists in placing the tissue in dilutions of alcohol of increasing strength, beginning with 60, 85, and 93 per cent., and absolute alcohol, in each of which the material remains for from twelve to twenty-four hours for pieces of ordinary size, and six hours for each concentration of alcohol where the block of tissue is small. As the introduction of tissue containing water into the solutions of alcohol gradually lowers the percentage, they must be occasionally tested with a hydrometer and brought up to the required strength.[2]

The water in the tissue occasionally contains substances in solution which are insoluble in alcohol, in which case the extracted solution sinks to the bottom of the dish. For this reason it is desirable to employ a cylindrical vessel of not too great diameter, which will give a high column of absolute alcohol, and to cover the bottom of the receptacle with wads of filter paper or cotton, or better still, to introduce a rack made of gauze or a perforated plate with feet, by means of which the preparation is kept in the upper layers of the alcohol, which possess the greatest concentration.

A ready means of preventing dilution of absolute alcohol is to cover the bottom of the dish with copper sulphate crystals which have previously been brought to a red heat and allowed to cool in a dry atmosphere. These crystals have the ability to take up the greater part of the water which goes into solution in the alcohol. As they take on the water they gradually become blue, when they must be removed and reheated. The blocks of tissue must be kept away from the crystals to prevent the adherence of particles of copper sulphate. Where the metal plate with feet is employed to keep the preparation in the upper portion of the alcohol, the crystals may properly be placed beneath it.

When the tissue has been dehydrated by passing it through the different concentrations of alcohol, it is placed in a clearing agent and embedded. (See page 33.)

The following are the most generally employed fixing and hardening solutions:

---

[1] For the action of different chemical fixatives on albumin and protoplasm, see A. Fischer, "Fixierung, Färbung und Bau des Protoplasmas." Jena, 1899 (Fischer).

[2] For the use of hydrometers in determining the strength of solutions of known composition, and for tables showing the density of dilutions of varying strength of chemicals used in microscopic technique, see Behrens, "Tabellen zum Gebrauch bei Mikroskopischen Arbeiten." Braunschweig, Harald Bruhn, 1898.

1. **Müller's Fluid**:

Potassium bichromate,	2.5
Sodium sulphate,	1.0
Aqua dest.,	100.0

The tissue to be hardened is immersed in a sufficient quantity of fluid, where it remains for several days. Where the material is taken from the central nervous system, weeks or months may be required to complete the hardening process. In the beginning the fluid must be changed daily. Where the hardening process extends over a longer period of time, every few days or once a week will suffice. The hardening process may be greatly expedited by placing the vessel containing the fluid and preparation in a thermostat.

Material which is to be cut with the freezing microtome must be washed for a short period of time and transferred for a few hours to a 5-per-cent. formaline solution, after which the manipulation is identical with that employed in the sectioning of material hardened in formaline alone.

If the material is to be embedded it must be thoroughly washed (with the exception of central nervous tissue), and treated with alcohols of increasing per cent. Material hardened in Müller's fluid is readily stained with alum carmine, lithium carmine, hæmatoxylin and methylene-blue. Müller's fluid is an excellent preservative for the red blood corpuscles, but gives less satisfactory results in the preservation of the nuclei.

2. **Ten-Per-Cent. Formaline Solution.** (**F.**) (**Equivalent to Four-Per-Cent. Formicaldehyde.**)—The solution is prepared by taking 10 parts of commercial formaline (40-per-cent. formicaldehyde in aqueous solution) and 90 parts of water.

This is one of the very best fixatives for ordinary routine purposes and possesses the great advantage of penetrating large pieces of tissue, acting quickly and effectively and giving excellent general results. Specimens hardened in formaline may be stained with all of the aniline dyes and ordinary nuclear stains and may be used wherever the success of the staining method does not depend upon the use of some special fixative.

3. **Combined Müller Formaline Solution.** (**Orth.**) (**M. F.**)—The solution is prepared by mixing 10 parts of commercial formaline with 90 parts of Müller's fluid. As the solution rapidly decomposes, it must be prepared directly before use and changed every few days. A ready method for preparing Orth's Müller-formaline solution consists in keeping two stock solutions, (*a*) Müller's fluid; (*b*) 10- to 20-per-cent. formaline solution; and mixing these two solutions in equal parts. A formaline solution stronger than 10-per-cent. must not be used in the preparation of this fixing solution, as its use is followed by a brown precipitate in the tissue, particularly in such portions of it as are infiltrated with blood or biliary coloring matter and may readily be mistaken for pigment which has been formed in the tissue.

Ten-per-cent. formaline and Müller-formaline solution possess the excellent qualities of fixing perfectly the red blood elements, and permitting the use of almost all nuclear stains and methods for the demonstration of bacteria (with a few exceptions; for instance, the pest bacillus). Albuminous fluids are coagulated by these solutions in homogeneous masses which may readily be mistaken for hyaline or colloid, an error which should be carefully guarded against.

The period of time required for fixation with either of these fixatives is from 12 to 24 hours at room temperature, or a shorter period when the vessel containing the fluid and preparation are placed in a thermostat. Small pieces of tissue may be sufficiently hardened in two or three hours at 36° C. Preparations hardened in 10-per-cent. formaline require no washing, but may be transferred directly to the alcohol.

All formaline solutions must be kept either in brown bottles or placed in a dark cupboard, as exposure to light causes oxidation of the formicaldehyde.

4. Alcohol.—When alcohol is employed as a fixative, ordinary 93-per-cent. ethyl alcohol gives the best results. Preparations are fixed in from 6 to 12 hours and the material is then transferred to absolute alcohol, where it remains until sufficiently hardened (6 to 12 hours). Alcohol produces rapid and sufficient hardening of the tissue for the simpler methods of sectioning and affords the best means of preparing material for cutting with the section razor. By this method rough sections may be cut from various portions of the hardened material with sufficient accuracy for purposes of orientation or diagnosis.

The disadvantage of the process consists in the marked shrinking which the tissue undergoes after treatment with alcohol and the imperfect manner in which the blood in the tissue is preserved. These disadvantages may be somewhat reduced by the addition to the 93-per-cent. alcohol, of formaline in the proportion of 1 to 10 (formaline-alcohol [F. A.]).

Preparations hardened in alcohol or formaline-alcohol cannot be cut with the freezing microtome until every trace of alcohol has been removed. The material should be transferred from the alcohol to a 10-per-cent. formaline solution, where it remains a few hours, after which it may be treated as if simply hardened in formaline solution.

5. Corrosive Sublimate. (Mercuric Bichloride.)—Bichloride of mercury is an excellent fixative and is employed in the following solutions:

(*a*) Saturated aqueous solution.

(*b*) Sublimate,	3
Glacial acetic acid,	1
Aqua dest.,	100

This solution produces less shrinking of the tissue than (*a*).

(*c*) Zenker's Fluid.

Sublimate,	5.	Dissolve with heat.
Potassium bichromate,	2.5	
Sodium sulphate,	1.	
Aqua dest.,	100.	

Before using add to the above:

Glacial acetic acid,	5.0

All material to be hardened in corrosive sublimate must be cut into slices not more than one or two millimeters in thickness. This precaution is necessitated by the poor penetration of the fixative. Fixation is followed by a period of thorough washing, 12 to 24 hours. Tissues treated with sublimate invariably contain deposits of precipitated mercury which take the form of minute black spheres in the free spaces of the tissue. The precipitate may be removed by treating the tissue with iodine (tincture of iodine) which is added to the 60-per-cent. alcohol in which the tissue is placed after washing. Sufficient tincture of iodine is added to give the alcohol a deep brown color. As the iodine unites with the mercury in the tissue the color of the solution gradually disappears. When the solution is no longer decolorized, the tissue may be transferred to a stronger alcohol, to which a few drops of iodine are likewise added. The further steps of hardening and embedding are the same as those following the use of other fixatives.

6. Flemming's Acid Mixture. This fixing solution consists of:

Glacial acetic acid,	1
Osmic acid, 2-per-cent. aqueous solution,	4
Chromic acid, 1-per-cent. aqueous solution,	15

7. Hermann's Fluid:

Platinum chloride 1-per-cent. aqueous solution,	15
Osmic acid, 2-per-cent. aqueous solution,	4
Glacial acetic acid,	1

The method of manipulation and treatment of the tissue after fixation is the same with both of these fixatives. Either solution must be prepared fresh before use, as it rapidly decomposes. These fixatives possess in common with all other mixtures containing osmic acid, the property of fixing and staining fat. Osmic acid forms a chemical combination with the fat, as a result of which it takes on a deep black appearance. Aside from being a fixative for the demonstration of fat, most osmic acid mixtures are excellent general fixatives, giving particularly excellent results in the demonstration of the finer structures of the cell (karyokinetic figures, etc.). The solutions do not penetrate well, for which reason the material must be cut into slices not more than one to two millimeters in thickness. Wads of paper or cotton may advantageously be placed in the solution to support the material to be hardened, so that the fixative may act upon all sides of the tissue. During the hardening process, which requires from 12 to 24 hours, the bottle containing the fluid must be placed in a dark closet, or may be encased in a dark receptacle. The hardening process is followed by thorough washing, 12 to 24 hours, and the usual second hardening with alcohol.

In carrying out the further steps of clearing and embedding the tissue, it is well to remember that the ordinary agents used in clearing the tissue, such as xylol and ether, dissolve the fat, therefore where it is considered desirable that the fat should remain in the tissue, it is best cleared with chloroform.

Preparations hardened in either Flemming or Hermann, may be cut with the freezing microtome after being treated with 10-per-cent. formaline solution for from 10 to 12 hours. Preparations hardened in mixtures containing osmic acid are difficult to stain and where it is considered desirable that the fat should remain in the tissue, safranin is perhaps the most reliable stain. Before staining the section, which is best fixed upon the slide with glycerine albumin, it is thoroughly washed in water to remove any excess of osmic acid, after which it must be thoroughly overstained with safranin and decolorized with alcohol. Where an osmic acid fixing solution has been used with the intention of demonstrating the nuclear structures, the osmic acid may be removed by oxidation. For this purpose the preparation should be placed in a dish containing commercial hydrogen peroxide (H_2O_2) full strength, where it remains until all black appearance has left the section and bubbles cease forming upon its surface,[1] when it is removed and washed for a few minutes in distilled water and may then be stained with any nuclear stain, the best for this purpose being iron hæmatoxylin.

8. Boiling Water.—Pieces of tissue may be quickly fixed by throwing them into a dish of boiling water where they are allowed to remain for about one minute. After removal the usual second hardening in alcohol follows, and the steps of embedding are the same as in all other methods of fixation. Boiling water has been used as a fixative for fluids containing albumin as in œdema of the lungs, demonstration of albumin in the tubules of the kidney in albuminuria, the action of heat causing the albumin in the fluids to be precipitated in the form of granular and stringy deposits.[1]

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[1] This valuable addition to our technique was suggested by Plimmer in his method for the demonstration of parasitic bodies in cancer.

9. **Dry Heat (Applied to Slides or Cover-slips).**—This method is employed in the examination of fluids, especially in examinations for bacteria and consists in spreading out, by means of a platinum needle, a sufficient amount of fluid to be examined upon a clean slide or cover-slip, or a small drop of fluid may be placed upon a cover-slip, a second cover-slip dropped upon the first and both drawn apart after the manner employed in making blood preparations. When prepared the slides or cover-slips are allowed to dry under a bell-jar, after which they are taken up with a forceps and passed three times rapidly through the flame of a spirit lamp or a Bunsen burner. By this means the material spread out upon the slide or cover-slip is fixed and ready for staining.

II. Decalcification of the Tissue.

Tissues containing either pathological or normal deposits of lime salts cannot be sectioned until they have been treated by some chemical which removes the deposits of chalk. This process is known as decalcification. Müller's fluid is the only fixative which possesses this quality and that to so small a degree as to be practically useless in the decalcification of any of the more difficult objects. The chemicals employed for this purpose are usually the mineral acids, either alone or in combination with some other agent, which is introduced with the intention of protecting the tissue from the severe action of the decalcifier. In spite of all precautions most of the mineral acids when applied either alone or in combination destroy the staining properties of the tissue. A marked exception to this statement is found in the case of sulphurous acid, as recommended by Paul Ziegler. The results obtained with this chemical are so much superior to all other methods recommended for the decalcification of tissue, that it seems unnecessary even to mention the proportions in which the other acids are employed. Sulphurous acid is employed in the ordinary commercial strength in which it is obtained (60 per cent. in water). The material to be decalcified is first hardened in a 10-per-cent. formaline solution or Müller-formaline solution. It is then transferred directly to commercial sulphurous acid full strength, in which three to four hours usually suffices for the decalcification ot ordinary blocks of tissue. Larger pieces of bone, such as the femur of a small child, usually require about two days. Material which has been treated with sulphurous acid for a longer period of time, still retains its staining qualities to a high degree. The period required for decalcification of all but the most difficult objects is so short that no alteration in the staining qualities of the tissue may be noted. Decalcification is followed by thorough washing and the usual treatment with alcohol before embedding.

III. Sectioning the Tissue.

1. **With the Section Razor.**—Tissues hardened in alcohol can be successfully cut with the section razor. When the student has developed a certain amount of skill in the use of the section razor, no difficulty will be experienced in cutting sections of sufficient thinness and size for almost all purposes of diagnosis. In one particular this method possesses advantages over all others;

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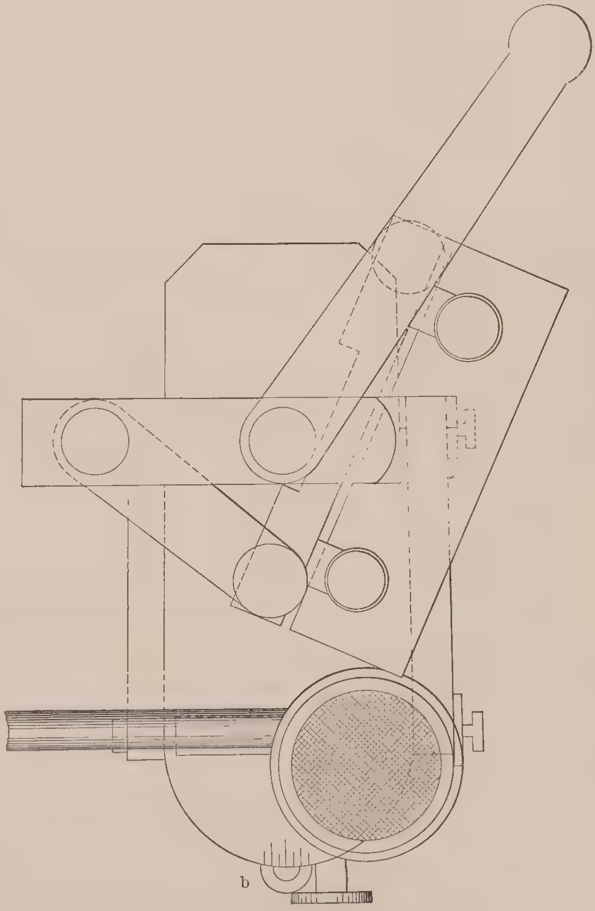
[1] Fixation of preparations by boiling has become more or less obsolete through the introduction of formaline which possesses the quality of precipitating and fixing the soluble albumins of the tissue.

the investigator has the privilege of selecting any point or surface on the block of tissue and removing sections which lie in widely different planes. For purposes of orientation this method is indispensable. The manner of holding and directing the razor when cutting is identical with that described for the manipulation of fresh material, except in this case the blade is kept constantly moistened in 60-per-cent. alcohol. The sections are transferred to 60-per-cent. alcohol, then to water and then stained. Thin and delicate objects, such as portions of the intestinal wall require support before they may be cut with the razor. The best means of affording the required rigidity consists in clamping the delicate material in a split block of amyloid liver (which has been previously hardened in alcohol), or alder pith. The sections are made in the usual manner, by cutting the supporting substance and the tissue at the same time.

2. With the Freezing Microtome.—The introduction of the freezing microtome in pathological-histological technique, has so simplified the sectioning of material for purposes of diagnosis and ordinary investigation, that no student or general practitioner need hesitate before the difficulties of the method. The freezing microtome in more or less crude form has been employed to cut fresh material, but the present extended use of the instrument is the result of the introduction of formicaldehyde as a fixative. The preparation is removed from the 10-per-cent. formaline solution (or Müller-formaline solution), and given a rapid washing before sectioning. Where the block of tissue is too thick a slice one or two millimeters in thickness may be cut from it, and this is placed in a few drops of water upon the surface of the freezing drum. It is then pressed in place with a needle or other instrument, and the freezing agent is allowed to act upon it until thoroughly frozen. Where the tissue does not freeze properly the suspicion must be awakened that it has by some means come in contact with alcohol. When this is the case the tissue is best returned to a 10-per-cent. formaline solution for a short period of time, when after thorough washing the freezing process may be resumed. A second cause for the improper performance of the

Fig. 2.

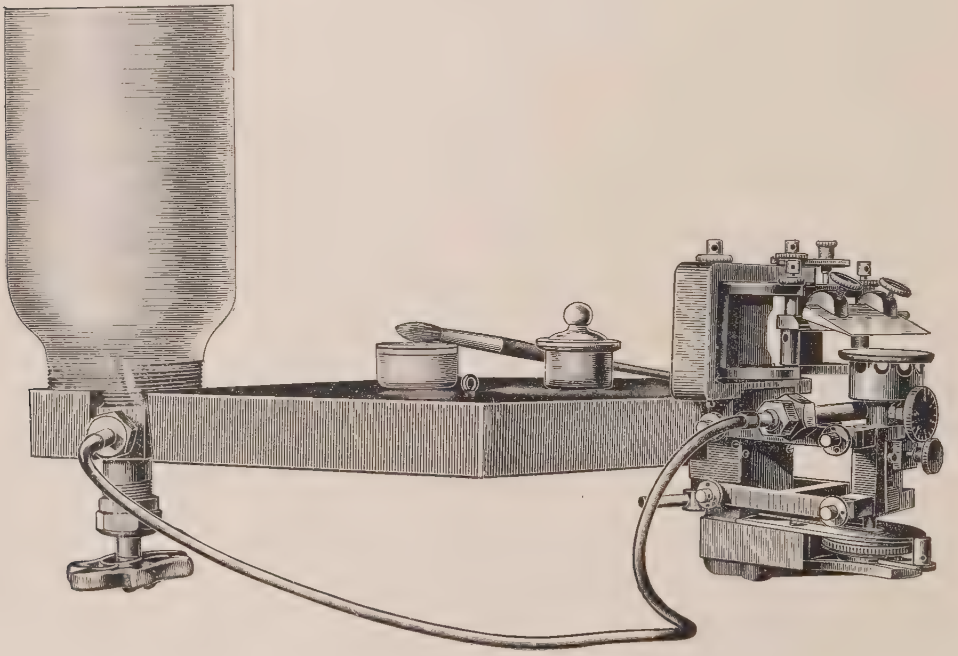


The Becker freezing microtome seen from above.

freezing apparatus is an accumulation of water upon the under surface of the freezing plate, as a result of which the plate becomes so thickened that the freezing process upon the upper surface is interfered with. Where the ether spray is used, constant irritation and interference is occasioned by clogging of the opening of the small ether jet. A fine brass wire should be kept in the opening when the jet is not in use.

The increased field of usefulness for the freezing microtome occasioned by the application of the formaline fixing method suggested the necessity for a better and more mechanical instrument than those commonly employed. Thus far the most popular instrument is that manufactured by Jung, and where economy is a factor, it may be recommended for the preparation of fairly good frozen sections. A better instrument and one devised at the suggestion of the authors is the Becker freezing microtome (Figs. 2 and 3). This microtome fulfills the requirements of a universal instrument, as excellent paraffine and celloidin sections may be cut with it, a quality which none of the inferior instruments possesses. It differs from all previous instruments by the direction which the knife takes in cutting (Fig. 2). The knife is supported by two arms placed at an angle to each other, which give it a peculiar sliding and pushing motion similar to that developed in cutting sections by free hand with the section razor. This motion is exceedingly favorable in cutting frozen tissues as it prevents the tearing which usually occurs when the knife is roughly pushed through the tissues, as in the Jung or Chathart instruments. The instrument is so constructed that pressure upon the handle (a) cannot influence the motion of the knife. The action of the microtome screw is automatic and the amount of elevation of the preparation at each motion of the knife is controlled by the lever (b). The thinness of the section is shown on a scale placed opposite the lever. A new freezing ap-

FIG. 3.



The Becker freezing microtome with the authors' freezing apparatus for carbon dioxide gas.

paratus has been devised for the use of carbon dioxide with this microtome. The instrument shown in Fig. 3 has been in use for over two years and has given admirable results. The connection between the cylinder of gas and the freezing table is made of flexible copper tube. The gas is controlled by a valve directly beneath the freezing table (Fig. 3). The valve of the cylinder is opened wide and the pressure rises in the tube. By opening and closing the valve beneath the table the plate of the freezing drum and the preparation upon it are frozen through without waste of gas or freezing of the connections, which is a constant occurrence where the cylinder and freezing table are connected with the ordinary rubber hose. When the freezing process is partly completed, the valve of the cylinder is closed, the gas remaining in the tube being sufficient to continue and complete the freezing of the preparation. The surface of the freezing table consists of a plate upon which is securely fastened a piece of gauze, by means of which the ice is held firmly upon the table. A small trough about the edge of the table prevents any fluid from finding its way to the under surface, thus delaying or preventing the freezing process. The entire freezing apparatus may be removed from the instrument and its place taken by a clamp suitable for paraffine or celloidin blocks. With this instrument frozen sections under 10 μ may be cut and paraffine sections as thin as 4 μ.[1]

The sections when cut are best taken from the knife with a camel's hair brush, and are placed directly in 60-per-cent. alcohol, where any adherent air bubbles are absorbed. The removal of the section from the knife is simplified when the brush is moistened with 60-per-cent. alcohol, but care must be taken that no alcohol falls upon the frozen tissue. The section is now transferred to a dish of water and then stained. If the section is very brittle it may be glued upon a slide or converted into a celloidin section in the following manner: From 60-per-cent. alcohol the section is placed in absolute alcohol for three minutes. When free from water it is drawn out on a *perfectly clean* slide, pressed flat with a piece of filter paper, and a solution of celloidin prepared as follows is poured over it:

Photoxylin, celloidin or gun cotton,	10
Alcohol absolute,	100
Ether,	500

Only sufficient celloidin to form a thin film is necessary, the remainder being allowed to flow back into the bottle. (Too much time should not elapse between the removal of the section from the absolute alcohol and the application of the celloidin solution; in no case should the section become dry.)

When the celloidin begins to set the slide is placed in a dish of water, where the film may be stripped up with the aid of a needle, the celloidin being first freed at the edges and raised carefully under the water. The detached celloidin film is trimmed into shape when it is ready for staining, or the section may be stained on the slide and detached later. The sections must not be treated with absolute alcohol but transferred from 93 per cent. to carbol xylol, or origanum oil; mount in balsam.

3. With the Sliding Microtome.—Various forms of the sliding microtome are in vogue. Excellent instruments of this type are manufactured by Becker in Göttingen, Jung in Heidelberg, Schanz in Leipzig, and a special form of the Minot sliding microtome is manufactured by Bausch & Lomb in America. For all purposes of the student the freezing microtome of Becker will give excellent results with paraffine or celloidin. The best universal large instrument is the sliding microtome of Becker. This instrument is so constructed that the slides upon which the carriage moves are of polished glass plates, as a result of which the ivory feet supporting the slide are not exposed to the amount of wear which occurs in the ordinary sliding microtome. This instrument is automatic, is furnished with a clamp which may be adjusted in all directions, has a mechanical hand motion and possesses all of the more complicated attachments which are desirable in a complete and elaborate instrument. Both paraffine and celloidin may be cut with equal facility and paraffine sections two or even one micro-millimeter in thickness may be made under favorable conditions. (See illustration, Part III.)

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[1] The Becker freezing microtome complete with the authors' freezing attachment is manufactured in America by the Spencer Lens Co. of Buffalo, N. Y. The price of the instrument has not been definitely fixed, but it will be in the neighborhood of $25.00, freezing attachment extra.

4. With the Ribbon Microtome.—With this form of instrument the knife is stationary and the preparation is moved. The best type of instrument is that devised by Minot. A perfected and more mechanical development of the Minot instrument has recently been made by Blake and is by all means the most perfect ribbon microtome in use. The advantage of the instrument consists in the rapidity with which thin paraffine sections may be cut and that these adhere and are run off in ribbons. In preparing serial sections the ribbon microtome is a great convenience, but it may hardly be considered as an instrument suitable for students or the general worker.

In the use of all the above described instruments the thinness of the section will depend largely upon the success of the embedding process and the quality and condition of the knife. (Embedding, see following chapter.) The knife must be honed and stropped in exactly the position it will occupy when clamped on the instrument. As the exact angle between the strop and the knife cannot be maintained during free-hand manipulation, a hollow cylindrical back is placed upon the knife in such manner that when the knife and back are held firmly upon the strop, the correct angle is obtained. (Fig. 4.)

FIG. 4.

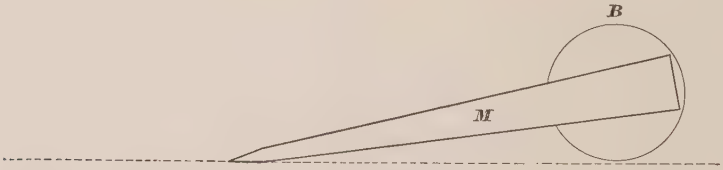


Diagram showing the position the knife should have during honing and strapping. *M*, knife; *B*, back.

Hard paraffine or celloidin must be cut with the knife placed at a very oblique angle, so that the knife is drawn through the paraffine and does not exert too great pressure upon the object. With soft paraffine this precaution is not necessary. The knife may even be placed at right angles to the long axis of the microtome and literally pushed through the paraffine. Oblong blocks should be placed with the long axis parallel to the carriage of the microtome. Paraffine sections should be prevented from rolling during the cutting process by the use of a camel's hair brush which is employed to keep the section flattened out upon the knife. The brush is applied immediately after the knife turns up the first corner of the section, and is held downward with just sufficient pressure to keep the section from rolling. Celloidin must be cut with the block and knife well moistened with 60- to 70-per-cent. alcohol. The paraffine or celloidin block must be firmly held in the microtome clamp. Any vibration of the object causes variations in the thickness of the section. During the process of cutting it is well to occasionally test the firmness of the block and tighten the screw which clamps it in place. After the section is obtained, whether paraffine or celloidin, it is transferred to 60-per-cent. alcohol, then to distilled water and then placed in the staining solution.

IV. Embedding.

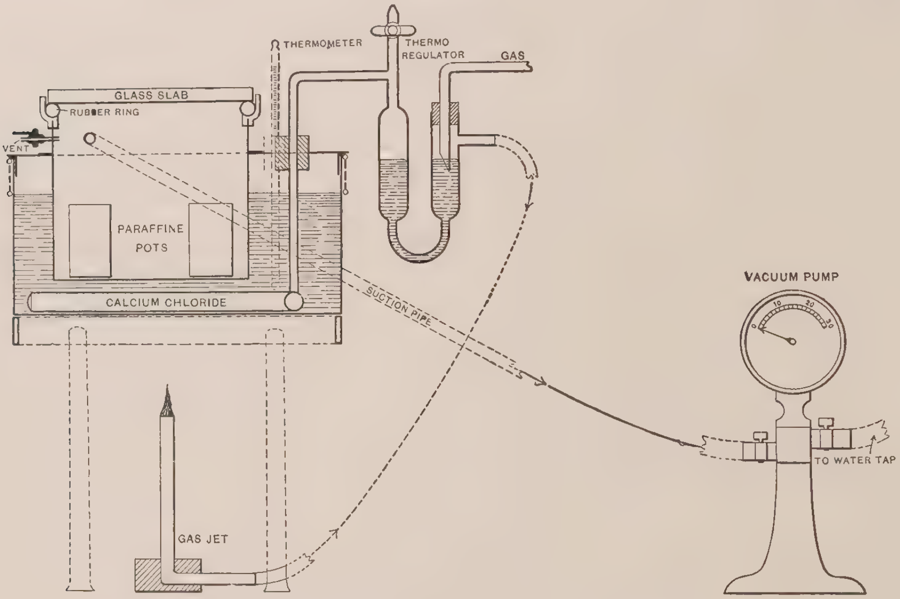
1. Paraffine Method.—Successful impregnation of tissues with paraffine is dependent upon complete dehydration of the tissue, which is usually affected by treating it with absolute alcohol (6 to 12 hours). After dehydration the tissue is placed in some paraffine solvent, such as xylol or chloroform and is allowed to remain in this until it becomes transparent. This treatment is known as clearing. The tissue must not be allowed to remain in the clearing agent longer than is required for the removal of the alcohol. Prolonged treatment, especially with xylol, causes contraction and distortion of the tissue, especially marked in the case of bone, tendons, and skin preparations, for which reason they are preferably cleared in chloroform. After the preparation is cleared in xylol (or chloroform), it is transferred to a saturated solution of paraffine in xylol (or chloroform) (2 to 3 hours), after which it is placed in melted paraffine, which is kept at the proper temperature in an oven or water-bath.

What is commercially known as hard paraffine has a melting point between 50 and 55° C. The so-called soft paraffine usually has its melting point between 45 and 50° C. By mixing these a paraffine with a melting point between 52 and 54° C. may be obtained. For general purposes this mixture will possess about the right density but the paraffine should be altered to suit the temperature at which it is to be cut, for which reason hard paraffine should be used in summer and in winter soft paraffine should be employed. In winter and when the paraffine is too hard, breathing upon the block will sufficiently warm the upper surface, or cutting the same in the neighborhood of a radiator or register will soften it to the required extent. In summer it is occasionally necessary to select a cool location when the paraffine has become too soft. The melting point of paraffine is higher than the point at which it solidifies; for this reason it may be melted over the free flame and then placed in an oven or water-bath, which is adjusted to a temperature which is just sufficient to keep it fluid.

The most convenient apparatus for embedding tissues in paraffine is a water-bath arranged with a thermo-regulator, which keeps it at a temperature of about 51° C. The bath proper is arranged with a heavy plate glass cover which rests upon a circular ring of rubber packing hermetically sealing the bath when the cover is in place. The pots of melted paraffine are placed in this bath. Through the sides of the bath are two openings, one fitted with a small valve to admit air, the other fitted with a nipple over which is slipped a heavy rubber tube connecting with a vacuum pump. When the block of tissue has been placed in one of the dishes of paraffine, the small valve is closed and the pump is brought into action, producing a partial vacuum within the paraffine-bath, as a result of which the diffusion between the paraffine xylol in the tissue and the melted paraffine occurs more rapidly than at ordinary atmospheric pressure. Two or three minutes suffices for the first treatment, after which the small valve in the side of the water-bath is opened, permitting the air to rush in, when the glass plate may be lifted and the block of tissue transferred to a fresh pot of paraffine. The procedure is repeated. The vacuum pump must be left in operation and air admitted to the water-bath only through the small vent in its side. If the vacuum pump is turned off before this is done, water will flow through the suction tube and injure the specimens. The details of the apparatus may be seen in Fig. 5.

Where the ordinary embedding oven is employed, the block of tissue, according to size, is placed for one half to one hour in a dish of fluid paraffine, then it is transferred to a second dish of melted paraffine to remove all traces of the clearing agent, then taken with a pair of warm forceps and placed between blocks upon a glass plate or embedded between pieces of metal which are so arranged as to enclose a series of square spaces.[1] A useful contrivance is that shown in Fig. 6, which is made of strips of metal placed at right angles to each other, one set containing slits in which the other strips fit, much after the fashion of the interlaced pieces of cardboard used in egg crates. These metal plates, after being properly arranged, are placed upon a glass plate, which forms the bottoms of the various cubical cavities, of which they form the sides.

Fig. 5.



Vacuum water-bath for embedding tissues in paraffine.

The metal plates and glass are thoroughly smeared with glycerine or vaseline before the paraffine is poured into them. The tissue is so placed that the surface from which the sections are to be cut rests upon the bottom of the receptacle. The operator then waits until the surface of the paraffine begins to harden, when the entire contrivance is immersed in a dish of cold water to insure rapid and homogeneous hardening of the paraffine. When thoroughly hardened the plates may be separated, and the paraffine blocks freed. An arrangement similar to the metal plates just described is formed by two L-shaped blocks of lead, which are placed together upon the glass plate. This contrivance has the disadvantage that only one block may be embedded at a time.

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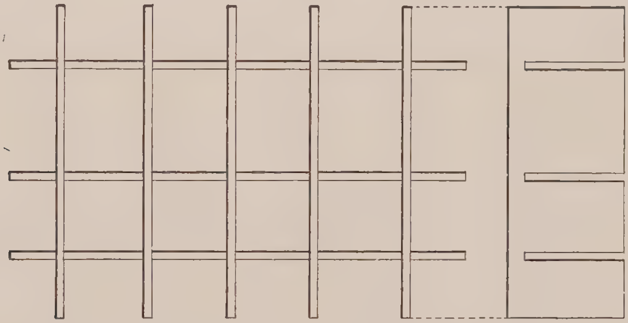
[1] When it occasionally seems desirable to hasten the process of embedding with paraffine, the method of Lubarsch may be recommended. One quarter to three quarters of an hour in absolute alcohol; renew the alcohol two or three times during this period. Transfer to aniline oil pure and place in paraffine oven for one quarter to one hour; transfer to xylol one half hour, change xylol two or three times until no yellowish discoloration of xylol results, then embed in paraffine.

When the paraffine block is taken from the water it is usually hard enough for sectioning and may be at once placed in the clamp of the microtome. If one wishes to economize, only sufficient paraffine to cover the tissue need be used, and after hardening, the thin block thus formed is mounted upon a square piece of wood by gluing it in place with melted paraffine.

Various errors in the method of embedding may be detected as follows: If the sections are too brittle, the paraffine has not been hardened with sufficient rapidity, or contains xylol. Either defect may be corrected by dissolving out the paraffine with xylol and repeating the embedding process, taking care that the trouble does not recur. If the knife does not enter the object properly and has a tendency to spring away from it, the tissue is either too hard, contains chalk, or has been embedded at too high a temperature. Where chalk deposits have remained in the tissue, they immediately ruin the cutting edge of the knife and must be removed. To permit of proper decalcification, the surrounding paraffine must be whittled away and the tissue returned to xylol until all paraffine is dissolved then placed in absolute alcohol to remove the xylol, and then treated with the decalcifying agent. When properly decalcified the tissue is reëmbedded.

FIG. 6.



Metal plates for forming paraffine blocks during embedding.

It is often necessary to attach paraffine sections to slides. Ordinarily this may be accomplished by floating the section upon a dish of water kept at 40° C., a temperature exactly sufficient to cause the section to spread out upon the surface of the water. This softens the paraffine so that when the section is drawn out upon a clean slide it adheres firmly to the surface of the glass by capillary attraction. The method is only successful when the slide is entirely free from fatty substance. After removal from the water, the slide with the adhering section must be dried in a thermostat at 36° C.

This method is not always successful when employed with tissue hardened in osmic acid. Such sections may be securely fastened upon slides by means of albumin-glycerine, which is prepared in the following manner: The white of an egg is beaten to foam, then filtered, and mixed with an equal quantity of glycerine and a small quantity of camphor to prevent decomposition. A very small amount of this mixture is rubbed upon the surface of a clean slide, a few drops of distilled water are placed over it and the section is floated upon the water, the slide is heated over a spirit lamp until the section spreads out upon the surface of the water, after which the water is poured from the slide and the section comes in contact with the albuminized surface. The slide is now placed in a thermostat at 36° for a short period of time to insure adhesion.

Before staining the paraffine may be removed from sections which have been attached to slides to insure greater rapidity in the action of the stain and a better differentiation. To remove the paraffine it is only necessary to immerse the slide in xylol; after a few minutes it is transferred to absolute alcohol, then to 90-per-cent. alcohol, from which it may be transferred directly to the stain without fear of the section becoming detached. (Strongly alkaline stains, as lithium carmine, commonly cause the section to become detached.) Paraffine blocks should be stored in a cool place and may be kept for an indefinite period of time.

Paraffine sections may be transformed into celloidin sections by the following method: The following solutions are prepared:

Solution 1:

Rock candy solution, 1 : 1,	300
Alcohol, 80 per cent.,	200
Dextrine solution,	100

The ingredients of the solution are mixed in the order in which they are put down. The dextrine should be of the yellow variety.

Solution 2:

Photoxylin, celloidin or gun cotton,	10
Alcohol, absolute,	100
Ether,	500

Solution 1 is poured upon a glass plate (for this purpose old photographic negatives may be employed after removing the film; 4 x 5 is a convenient size), which has been thoroughly cleaned with soapsuds and warm water. The sugar solution must be made to cover the entire plate of glass by tipping it from side to side and allowing the excess to flow back into the bottle. Upon this slide the dry paraffine sections are spread out in the order in which they are cut, if they are to be used in series. The glass plate with the sugar solution and sections is placed in a thermostat at 36° C. until the sugar is perfectly dry. It is then placed in a porcelain developing tray and covered with xylol to remove the paraffine. The sections adhere to the sugar upon the glass. The plate is then transferred to a second dish and covered with absolute alcohol. All xylol must be removed from the plate. The plate is taken from the alcohol and the photoxylin solution (No. 2) is poured over it, the excess being again returned to the bottle. When the film of photoxylin begins to set, the glass plate is immersed in a dish of water when the sugar solution is dissolved from beneath the film of celloidin to which the sections adhere. The film may now be loosened by scratching through it at the edges of the plate on three sides, allowing it to adhere to the plate on the fourth side. The sheet of celloidin and attached sections may now be treated by any of the ordinary staining methods. The sections need not be cut apart until ready for mounting. This method gives the most reliable results when the various steps are properly carried out. When the sugar solution fails to spread out upon the glass plate, this indicates that the glass has not been thoroughly cleaned. After the celloidin has been poured upon the plate and it has been placed in a dish of water, if the celloidin shrinks it indicates that it has not sufficiently hardened and when it turns white, this is due to the presence of xylol.

2. Celloidin Method.—The celloidin method may be carried out without the aid of heat, and is distinguished from the paraffine method by the embedding substance remaining permanently in the sections, for which reason it is employed where the tissue is frail and especially thin sections are not required.

It is not possible to cut sections thinner than 5 μ from material which has been embedded in celloidin. The solutions of celloidin used in the impregnation of tissue are prepared by dissolving commercial celloidin or gun cotton in a mixture of ether and alcohol, equal parts. Three solutions of increasing thickness should be prepared. The first should have a consistence about equal to ordinary collodion; the thickest about that of thick syrup, and the second a consistency between these two. The tissue to be embedded is removed from the absolute alcohol and placed in a mixture of ether and alcohol, equal parts for 24 hours, after which it is transferred to the thin solution of celloidin 24 hours, the second solution of celloidin 24 hours, and the thick solution 24 hours, or, if necessary, a longer period of time. A suitable receptacle for the solidifying celloidin may be made by folding writing paper into the required form, allowing the celloidin to harden in this receptacle until the surface is slightly glazed and then placing the whole in 70- to 80-per-cent. alcohol, where it should remain until of the proper density. When sufficiently hard the block is removed from the paper box and fastened upon a block of vulcanized fiber, being glued in place with celloidin solution. Before and after sectioning the celloidin block must be kept in 60- to 80-per-cent. alcohol. Remnants of celloidin or gun cotton may be used a second time, after thorough drying, by redissolving in ether-alcohol.

Celloidin is sectioned with the knife placed at an oblique angle, the block and surface of the knife being thoroughly moistened with 60-per-cent. alcohol. The section when cut is removed from the blade of the knife with a camel's hair brush moistened in 60-per-cent. alcohol and transferred to a dish containing the same. It is occasionally necessary to remove the celloidin from the section before staining, which is accomplished by passing it through alcohols of increasing strength to absolute alcohol, then placing it in a mixture of alcohol and ether, equal parts, in which the celloidin is dissolved, after which it is returned to absolute alcohol, then carried through the thinner alcohols in decreasing order to 60-per-cent. alcohol, when it may be transferred to water and stained. This step is occasionally required when sections embedded in celloidin are to be stained with aniline dyes. Where the celloidin section has been stained with aniline stain, the section may be cleared in oil of cloves, which dissolves the celloidin. Where it is considered desirable to retain celloidin in the section, which is usually the case, agents which dissolve the celloidin should be avoided (absolute alcohol, oil of cloves, etc.).

Serial sections from material embedded in celloidin may be made after the method of Bumpus:

1. The block of tissue is stained in a solution of alum carmine of the following composition:

Carmine,	2
Alum,	4
Aqua dest.,	400

Evaporate this solution over a water-bath to 200 parts, after which add 5 drops of carbolic acid.

Small pieces of tissue 3 millimeters or under, are sufficiently impregnated with this staining fluid in 24 hours. Larger fragments of tissue must be allowed to remain in the stain for a longer period, occasionally for weeks. Overstaining does not result. In place of alum carmine, alum hæmatoxylin may be employed, in which case the fragments of tissue are placed for 24 hours in a dilute solution of alum hæmatoxylin. (Mayer.) If the tissue is overstained, it should be placed in a 1-per-cent. alum solution for 24 hours.

2. Wash 12 to 24 hours in running water.
3. Alcohols of increasing per cent., 24 hours each.
4. Absolute alcohol 24 hours.
5. Absolute alcohol and ether, āā 24 hours.
6. Pass through the three solutions of celloidin, which will require several days.
7. Place in boxes made of writing papers, cover with celloidin, and place under bell glass to solidify.
8. Place in chloroform to complete hardening.
9. Transfer the block to a mixture of chloroform and castor oil, āā (oil of thyme or oil of lavender). In this solution the celloidin becomes transparent.
10. Cut, moistening the knife with the oil and chloroform mixture.
11. Fasten to slides.
12. Dry slides under bell-jar one quarter to half an hour.
13. Remove superfluous fluid from sections, embed in Canada balsam.

Celloidin sections may be attached to slides which have been cleaned with alcohol, by spreading them out carefully upon the surface and passing them firmly upon the slide with a piece of blotting paper. A ready means of transferring the section is to spread it out upon the microtome knife, press a clean piece of tissue paper upon it, to which it will adhere, transfer it to the slide, press it into place and then remove the strip of paper. The section must not be allowed to dry. Where the section does not adhere properly to the slide, or where fixation is desired, the section should be treated with ether vapor, which softens the celloidin and causes adherence to the slide. The vapor is applied by pouring it from a bottle half filled with ether, following along the edges of the section until they flatten down, when the slide and section should be placed in 80-per-cent. alcohol to re-harden the celloidin. A little practice in the application of the ether vapor will overcome all difficulties.

V. Staining the Preparations.

All staining agents must be kept in well-stoppered glass bottles and before use should be filtered to remove all particles of dirt, precipitates, bacteria, or moulds which may have accumulated in them. For this reason it is advisable to acquire the habit of filtering, as it is required, the portion of stain to be used. A convenient wire support, in which is placed a small funnel and a properly folded filter paper, should be kept upon the student's desk, and the necessary amount of stain filtered into a shallow dish when needed. A separate piece of filter paper is kept for each stain, and may be used repeatedly, so long as there are no holes in it. When the precaution of filtering the stain is not taken, the student will be constantly disappointed by finding masses of precipitated coloring matter, dirt, etc., marring the appearance of his preparations. A shallow porcelain dish, the size and shape of the ordinary butter plate is a convenient receptacle for the stain. Glass dishes of the same size and depth are useful where the stain is of dark color, or not very transparent, in which case the recovery of the section is facilitated by holding the dish over a white surface from which light is reflected. Where sections have been glued upon slides, suitable dishes must be provided in which the slides may be immersed. Sections must be transferred from one dish to another by means of a section lifter, the excess of fluid being drained from the preparation by placing one corner of the lifter upon a convenient piece of filter paper. Sections embedded in any of the various ways already described, may be removed directly from the microtome knife and placed in the staining solution. In the case of paraffine sections it is not necessary to remove the paraffine, the sections staining quite as well, but requiring a somewhat longer period of time. The paraffine in the section serves the purpose of protecting it from injury and lessens the difficulties of manipulation. During the staining process the paraffine sections are floated upon the surface of the stain and covered with a small piece of filter paper to insure action of the stain upon both sides of the section. Sections which are fastened upon slides do not require the protection of the paraffine, which may be removed, thus shortening the staining time.

1. Nuclear Stains.[1]—The stains under this heading possess the quality of staining the nuclei of the tissue and do not, or but faintly, stain the protoplasm. In the majority of cases the simple nuclear stain will suffice for all diagnostic purposes.

(*a*) **Carmine Solutions.**—(*α*) **Alum Carmine.**

FORMULA.—1 gram of pure carmine is rubbed in a mortar together with 5 grams of alum, the whole dissolved in 100 cc. hot water and boiled over a water-bath for 10 to 20 minutes. When cool, filter. Bacteria and moulds develop readily in this solution, for which reason it must be filtered before use. Material which has been hardened in alcohol, formaline, Müller-formaline, Müller's fluid or by cooking are readily stained with this solution.

METHOD.—Sections are placed for from 10 to 15 minutes in the staining solution. Overstaining does not readily occur. Rinse in distilled water, mount in glycerine, or transfer to alcohol, xylol and mount in balsam.

(*β*) **Lithium Carmine.** FORMULA.—2.5 grams of pure carmine are dissolved in 100 cc. of a cold saturated aqueous solution of lithium carbonate. This solution is brought to the boiling point, and after cooling, filtered. Suitable for material which has been hardened in formaline, Müller-formaline, Müller's fluid, alcohol, or by cooking.

METHOD.—Sections remain from 1 to 15 minutes in the stain. In this they become a deep red and are then differentiated in acid alcohol (may require from a few seconds to several hours). The nuclei must be sharply differentiated from the protoplasm. Absolute alcohol, xylol, balsam.

(*b*) **Hæmatoxylin Solutions.**—A great variety of hæmatoxylin solutions have been recommended. The following are the most reliable:

(*α*) **Bœhmer's Hæmatoxylin:**

Solution *a*.	Hæmatoxylin crystals,	1 gram.
	Absolute alcohol,	10 cc.
Solution *b*.	Ammonia alum,	20 grams.
	Aqua dest. (hot),	200 cc.

On cooling, filter. After 24 hours *a* and *b* are poured together and allowed to remain for eight days in an open, wide-mouthed vessel exposed to the air and light, when the solution is filtered and is ready for use.

(*β*) **Delafield's Hæmatoxylin:**

Hematoxylin crystals,	4 grams.
Alcohol (95 per cent.),	25 cc.

When the ingredients are dissolved the above is added to 400 cc. of a saturated aqueous solution of ammonia alum. Expose the mixture in an open-mouthed vessel to light and air for several days. Filter, and add the following:

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[1] All stains should be those prepared by Gruebler, Leipzig, which may be obtained from any dealer in microscopical supplies.

Glycerine,	100 cc.
Alcohol (95 per cent.),	100 cc.

Allow the mixture to remain in the light until it has become of a sufficiently deep color, when it is filtered and kept in a tightly stoppered bottle.

(γ) **Erlich's Acid Hæmatoxylin:**

Solution *a*.	Hæmatoxylin,	2 grams.
	Absolute alcohol,	60 cc.
Solution *b*.	Glycerine,	60 parts.
	Aqua dest.,	60 parts.
	Glacial acetic acid,	3 parts.

Ammonia alum to be added till saturation.

Solutions *a* and *b* are then mixed and the mixture allowed to ripen in an open wide-mouthed bottle for eight days, whereupon it is filtered and placed in a tightly stoppered bottle.

(δ) **Mayer's Hæmalum:**

Solution *a*.	Hæmatine,	1 part.
	90-per-cent. alcohol,	50 parts.

Dissolve by heat.

Solution *b*.	Alum,	50 parts.
	Aqua dest.,	100 parts.

Mix *a* and *b* and, after cooling, filter.

(The addition of glacial acetic acid in the proportion of 2 per cent. produces acid hæmalum, which is said to differentiate somewhat more sharply than the ordinary hæmalum.)

Material which has been hardened in alcohol, sublimate, Müller-formaline or Müller's fluid, may be stained with any of the above solutions of hæmatoxylin.

METHOD.—As all hæmatoxylin solutions increase in strength with age, no definite rule may be laid down as to the length of time required for staining. Older solutions are best diluted with 1-per-cent. aqueous solution of alum. The solution should be so diluted that a proper stain is obtained in from 3 to 10 minutes, after which the preparation is thoroughly rinsed in distilled water. The longer the sections remain in the water after staining (1 to 2 hours) the more beautiful the differentiation becomes.

Overstaining is easily corrected by treatment with acid alcohol prepared as follows:

Hydrochloric acid,	1 part.
Alcohol (70 per cent.),	100 parts.

The section remains in the acid alcohol from one-half to several minutes and changes under the action of the acid to a pale red color. When sufficiently decolorized the section is thoroughly rinsed and allowed to remain in a dish of water for from one quarter to several hours, during which time it regains its original blue color. The sections must be immersed in the water and not floated upon the surface.

All hæmatoxylin solutions with the exception of alum hæmatoxylin readily overstain the tissue. The length of time which the section may remain in the solution must be accurately determined and even with alum hæmatoxylin, where the preparation is exposed to the action of the stain for an indefinite period of time, overstaining may result.

Sections may be embedded in glycerine, or transferred to alcohol, xylol and mounted in balsam.

(c) **Aniline Stains.** (α) **Methylene-blue.**

FORMULA.—The staining solution is prepared by dissolving one half to one gram of methylene-blue in 100 cc. of distilled water, bringing the solution to the boiling point and filtering.

METHOD.—Sections are placed in the staining solution from 5 to 10 minutes. Wash in water. All aniline dyes have a tendency to diffusely stain the tissue, and require differentiation in absolute alcohol, which removes the color from the protoplasm, but not from the nuclei or bacteria. Where the preparation is overstained, a more energetic decolorization or differentiation may be obtained by the use of dilute acetic acid, after which the section must be again treated with absolute alcohol; clear in xylol and mount in balsam.

Material which has been hardened in alcohol, Müller's fluid, formaline or Müller-formaline gives excellent results with methylene-blue.

(β) **Safranin.** FORMULA.—This stain is used in the form of a 1- to 2-per-cent. aqueous solution, or:

Safranin,	1.0
30-per-cent. alcohol,	100.0
Aqua dest.,	200.0

METHOD.—Sections remain from onehalf to several hours in the solution. Differentiate with acid alcohol. Transfer to absolute alcohol, which causes further differentiation. When properly decolorized, only the nuclei and nuclear structures are stained. Clear in xylol and mount in balsam.

Safranin gives excellent results with material hardened by all of the ordinary methods (same as methylene-blue), but is especially useful as a stain for material which has been hardened with any osmic acid fixative, especially when the osmic acid is left in the tissue.

2. Double or Contrast Stains. (α) **Orth's Picro-carmine.** FORMULA.—1 part of lithium carmine (dissolve 2.5 grams of pure carmine in 100 cc. of a cold saturated aqueous solution of lithium carbonate, bring to the boiling point, allow to cool and filter) is mixed with two to three parts of cold saturated aqueous solution of picric acid. The proportion of this mixture, 1 part of lithium carmine to 2 or 3 of picric acid, is not arbitrary. Where the preparation is too yellow, the proportion of picric acid must be reduced by adding more lithium carmine to the solution, and where the reverse is the case and the tissue is too red, the addition of a suitable amount of pure picric acid will correct the defect.

METHOD.—Sections are stained with the solution 10 to 15 minutes. Transfer to and differentiate in acid alcohol (1 part hydrochloric acid to 100 parts 70-per-cent. alcohol) until the section takes on a pale red color. Dehydrate in absolute alcohol, clear in xylol, mount in balsam.

Absolute alcohol has a tendency to remove all the picric acid from the tissue, a defect which may be corrected by dissolving a few crystals of picric acid in the absolute alcohol which is employed for dehydration of sections stained by this method. Picric acid stains the protoplasm of the cells, striated and non-striated muscle fibers, hyaline and colloid material, horny substance of the epidermis and fibrin, giving them all a bright yellow color. Nuclei are stained red by the carmine.

Another method of applying carmine and picric acid in combination consists in staining the section with lithium carmine, differentiating in absolute alcohol and dehydrating in absolute alcohol in which crystals of picric acid have been dissolved, the action of the picric acid alcohol being controlled by the length of time the preparation is allowed to remain in it.

Picro-carmine is suitable for tissues which have been hardened in alcohol, formaline, Müller-formaline, or Müller's fluid.

(*b*) **Hæmatoxylin-eosin.** FORMULA.—Eosin is employed in the form of eosin-alcohol, which is prepared by adding a few drops of concentrated solution of eosin in absolute alcohol to the dish of absolute alcohol in which the sections are dehydrated. The strength of the eosin-alcohol, and the length of time which the section remains in it is determined by practice. Eosin stains protoplasm and muscle a deep red color.

METHOD.—The section is stained with hæmatoxylin, thoroughly washed in water, and transferred to eosin-alcohol where it is allowed to remain until it takes on a pale red color, when it is transferred to xylol and mounted in balsam.

This method is suitable for tissues which have been hardened in alcohol, sublimate, formaline, Müller-formaline or Müller's fluid. A most beautiful eosin reaction may be obtained with tissues hardened in sublimate, by the method of Guipel. The section is stained in Delafield's hæmatoxylin, then placed in a dish of saturated alcoholic solution eosin one part, water one part. Allow the section to overstain, and decolorize by washing in changes of water until no more color is removed. Sections which have been hardened in formaline should be placed for a few moments before staining in a sublimate solution, which will assist the reaction.

(*c*) **Methylene-blue Eosin.**—Eosin may be used as a counter stain for sections which have been stained with methylene-blue, the sections being transferred, after washing in water, to eosin-alcohol, where they remain until they take on a pale red tinge. Transfer to xylol, mount in balsam. Material which has remained for a longer period in Müller's fluid is unsuitable for this method.

(*d*) **Van Gieson's Stain.** FORMULA.—Concentrated aqueous solution of picric acid, 100 parts; concentrated aqueous solution of acid fuchsin, one to three parts. The two are mixed together, filtered and the solution is ready for use.

METHOD.—The sections are stained with Delafield's hæmatoxylin, washed in water, and transferred to the picric acid fuchsin solution, where they remain from three to five minutes. Wash in water for from one half to two minutes. Dehydrate and differentiate in absolute alcohol, xylol, balsam. Washing and dehydration of the section will influence the yellow or red which remains in the tissue and the relative intensity of the hæmatoxylin. Fuchsin is removed by a longer treatment with water, picric acid by absolute alcohol. The manner in which the tissue has been hardened and the length of time employed for the various steps during staining will determine the relative intensity of the different colors. In a properly stained specimen the nuclei should be reddish brown to violet; connective tissue brilliant red; non-striated muscle fibers yellow; hyaline and amyloid dark red; colloid orange or yellowish red.

VI. Clearing and Mounting the Preparation.

The stained section requires treatment with certain agents whose function is to render the tissue more or less transparent, thus subordinating the structural outlines of the tissue and increasing the prominence of the color picture. This step is known as clearing. In some cases the clearing agent is a suitable mounting medium.

1. With Glycerine.—Glycerine is used as a clearing and mounting medium for sections cut with the freezing microtome, or the section razor. The stained or fresh section is transferred by means of a section lifter from the water in which it has been washed, to a slide upon which it is carefully spread out. The superfluous water is removed with a piece of filter paper, and a few drops of glycerine is placed upon the section which is then covered with a cover-glass. If the section is floated in a dish of sufficient size, it may be drawn directly upon the slide and treated in the manner above described, thus dispensing with the use of the section lifter. The paraffine must be removed from paraffine sections before they are embedded with glycerine. This is accomplished by treating them with xylol, passing them through absolute alcohol, then through the various per cents. of dilute alcohol in decreasing order, rinsing in water and spreading them out upon a slide and covering with a few drops of glycerine. Glycerine is only suitable for sections which have been stained with alum carmine or hæmatoxylin. Aniline stains pass into solution in glycerine and should not be used as clearing and embedding material for sections thus stained.

2. With Potassium Acetate (Saturated Aqueous Solution).—Potassium acetate is employed for sections which have been stained with aniline dyes and which may not be embedded in glycerine. It is used in exactly the same manner as that described for mounting specimens in glycerine. Preparations mounted in either of these media rapidly dry out. When it is thought desirable to preserve the preparation for a greater length of time, the evaporation may be prevented by hermetically sealing the space between the edges of the cover-slip and the slide. Melted wax is employed for this purpose and is easily applied with a brush, taking care to remove all glycerine or potassium acetate from the edges of the cover-slip and the adjacent portions of the slide with a cloth moistened in alcohol. Having fixed the four corners of the slip by applying drops of wax to them, the edges are carefully painted over with the melted wax. Instead of melting the wax in a dish a paraffine taper may be used.

3. With Xylol and Ethereal Oils.—Xylol is used as a clearing agent for preparations which have been embedded and stained and which are to be mounted in balsam.

(*a*) **Paraffine Sections, or Sections of Hardened Material which have been cut on the Freezing Microtome and Stained.**—All sections which are to be embedded in xylol are placed in absolute alcohol until thoroughly dehydrated, which requires from one half to two minutes, after which they are transferred to xylol where they remain until they become perfectly transparent. If the section shows cloudy or grayish spots after treatment with xylol these indicate that it still contains water, and must be returned to absolute alcohol to remain a longer period of time, before again mounting in xylol. In order to detect these spots the preparation is best placed against a black background when the portions of the section containing water may be readily detected. Xylol dissolves the paraffine from the section.

(*b*) **Celloidin Sections.**—Celloidin sections must not be placed in absolute alcohol, which softens the embedding material, and are therefore dehydrated in 93-per-cent. alcohol. As this treatment does not remove all the water from the sections, they must be cleared in some agent which is not too sensitive to the presence of small amounts of water. In place of xylol, the section should be cleared in

(*a*) Origanum oil, or

(*b*) Carbol-xylol :

Xylol,	3.0
Carbolic acid crystals,	1.0

After treatment with either of these clearing agents, the sections must be perfectly clear and free from gray spots, which indicate the presence of water. When these appear the section must again be treated with 93-per-cent. alcohol. Sections which have been cleared with xylol, carbol-xylol or origanum oil are usually mounted in Canada balsam. To embed in balsam, the section is transferred to a slide (where it has not been previously fastened to the slide, in which case the slide and section have been passed through the agents for dehydrating and clearing), the superfluous clearing agent is removed with a scrap of filter paper, a small drop of Canada balsam dissolved in xylol is placed upon the slide and a cover-glass of sufficient size to cover the preparation is laid upon it. The cover-slip is now carefully pressed down, taking care not to exert too much pressure, as the section is readily broken. The superfluous balsam which flows out at the edges of the slide must be carefully wiped away with a cloth moistened in xylol. A too thin solution of Canada balsam must not be used, or after the evaporation of the xylol the preparation will not contain sufficient balsam to preserve it. After being properly mounted and the edges carefully wiped, the slide may be placed in a drying oven at a temperature of 36° or even higher, where it should be allowed to remain for from a few hours to a few days to hasten the fixation of the balsam. When the balsam is thoroughly dried the preparation may be handled with impunity.[1]

VII. Scheme for the Microscopic Examination of Pathological Material.

The simplification of pathological-histological technique resulting from the introduction of the freezing microtome, should place it within the power of every practising physician and student to carry out a routine examination of all the pathological material which comes to hand. Such material may be manipulated according to the following scheme:

1. Fresh examination of teased tissue or sections.
2. Remove small pieces of material, not above one or two millimeters in thickness, from the interesting portions of the organ or tumor, and place them in a sufficient quantity of 10-per-cent. formaline or Müller-formaline solution.
3. Place the bottles containing the material to be hardened in a thermostat or in some warm locality, as in the neighborhood of a stove or gas-burner (6–12–24 hours).
4. Cut the sections with a freezing microtome, transfer to 60-per-cent. alcohol.
5. Stain in alum carmine or alum hæmatoxylin.
6. Rinse in distilled water.
7. Stain, mount and examine in glycerine, or,
8. Transfer from water to absolute alcohol for dehydration (1 to 15 minutes).
9. Clear in xylol (one half to one minute).
10. Embed in Canada balsam.

This method will suffice for the investigation of all pathological material of importance or interest to the practising physician or student. Where the student wishes to carry the investigation further the material may be embedded in paraffine, in which case a modification of the steps after No. 3 will be necessary:

4. Wash the hardened tissue for from one half to one hour in running water.
5. Place in 60-per-cent. alcohol (according to size of the material, 6 to 12 hours).
6. Same length of time in 93-per-cent. alcohol.
7. Like period of time in absolute alcohol.

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[1] For the treatment of specially stained sections which are decolorized by xylol, such as Weigert's fibrin stain, see appropriate heading.

8. Place in xylol until transparent ($\frac{1}{2}$ to 3 hours).
9. Place in paraffine xylol (2 to 3 hours.)
10. Transfer to fluid paraffine in water-bath or paraffine oven (one half to one hour).
11. Place in second dish of fresh fluid paraffine (2 to 3 hours).[1]
12. Transfer the block of tissue to a salt cellar containing fluid paraffine or embed between embedding plates.
13. When the surface of the paraffine is cool immerse the block in water to insure rapid hardening.
14. Cut with the microtome and transfer the sections to 60-per-cent. alcohol.
15. Transfer to water and stain with alum carmine or alum hæmatoxylin (10 to 30 minutes).
16. Wash sections in water.
17. Dehydrate in absolute alcohol (several minutes).
18. Clear and remove paraffine from the section in xylol (one half to several minutes.)
19. Mount in Canada balsam.

Where the material is of a character which requires embedding in celloidin, this may be carried out by substituting the following steps for those which occur after No. 7 of the paraffine method :

8. Transfer to ether alcohol āā, for 24 hours.
9. Place in thin celloidin solution 24 hours.
10. Transfer to thick celloidin solution for from 24 hours to several days.
11. Fasten upon blocks of vulcanite fiber and harden celloidin in 80-per-cent. alcohol (12 to 24 hours).
12. Cut with the microtome.
13. Stain in alum carmine or alum hæmatoxylin (5 to 15 minutes or longer).
14. Wash in water.
15. Dehydrate in 93-per-cent. alcohol.
16. Clear in origanum oil, or carbol-xylol.
17. Embed in Canada balsam.

VIII. Methods for the Preservation of the Natural Colors of the Tissues.

It is occasionally desirable to preserve the color in macroscopic specimens for future reference. This can be accomplished without destroying the utility of the material for purposes of microscopic examination by hardening it in the special solutions about to be named. The procedure should be carried out where the student wishes to refer to the original material for purposes of demonstration or control. The tissue is hardened in formaline to which are added various salts, and in this takes on a grayish appearance. After being sufficiently hardened, the necessary time depending upon the size of the preparation and its consistence, the specimen is transferred to weak alcohol, in which it recovers its original color, when it is transferred to a preserving fluid in which it is kept. Large organs, as the liver, are hardened with difficulty, to overcome which the fixative may be injected into the large vessels, or small slices of the organ showing the principal alterations of tissue should be subjected to treatment. Occasionally a thin layer of the tissue will serve the purpose, in which case the transparency of the tissue is more apparent and the colors brighter. Preparations which have been kept in the preserving fluid for a period of time, and have lost their color, may be restored by returning them to alcohol, and where the alterations of the tissue are shown in cross section, a fresh surface may be cut. Plenty of fixing solution must be employed, and the preparation must be placed in the position which it should occupy after hardening. It should be supported upon wads of paper or balls of cotton. After hardening in the formaline salt solution, portions of the tissue may be removed for microscopic examination, and these may be transferred to thin formaline solution and cut on the freezing microtome, or transferred to alcohol and embedded in paraffine. The length of time required for fixation in the case of small specimens is about 24 hours; larger organs requiring from two to eight days. The length of time during which the preparation should be exposed to the action of the alcohol varies from one to six hours. At no time during the steps of the process is the specimen washed with water. Both the formaline salt-fixing solution and the alcohol may be used repeatedly. When the solutions have been used a great number of times it is occasionally desirable to finish both steps by placing the organ for a short period in fresh solutions. The most satisfactory methods for the preservation of the natural colors in pathological material are the methods of:

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[1] Where the student possesses the vacuum water-bath shown on page 34, the length of time the preparation remains in the paraffine is reduced to a few minutes.

1. Melnikow-Raswedenkow:[1]

(*a*) Fixing Solution:	Sodium acetate,	4.0
	Potassium chlorate,	0.5
	Formaline,	10.0
	Aqua dest.,	100.0
(*b*) 93–95-per-cent. alcohol.		
(*c*) Preserving fluid:	Glycerine,	20.0
	Potassium acetate,	15.0
	Aqua dest.,	100.0

2. Jores:[2]

(*a*) Fixing solution:	Sodium chloride,	1.0
	Magnesium sulphate,	2.0
	Sodium sulphate,	2.0
	Aqua dest.,	100.0
	Formaline,	5 to 10 parts.
(*b*) Alcohol.		
(*c*) Preserving fluid; glycerine and water āā.		

3. Kaiserling.[3]

(*a*) Fixing solution:	Formaline,	800 ccm.
	Aqua dest.,	4,000 "
	Potassium nitrate,	45 g.
	Potassium acetate,	85 g.
(*b*) Alcohol, 80 per cent to 95 per cent.		
(*c*) Preserving fluid:	Aqua dest.,	2,000 ccm.
	Potassium acetate,	200 g.
	Glycerine,	400 g.

IX. Special Staining Methods.

1. Nuclear and Protoplasmatic Structures. (*a*) **Nuclear Structures.**—The nuclear figures found during the progress of karyokinesis are usually demonstrable after fixing and hardening the tissue in alcohol, sublimate or formaline and staining with hæmatoxylin or Gram's method. (See pages 39 and 64.)

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[1] Centralblatt f. path. Anat., Bd. XI., 1900, S. 151.

[2] Ibidem, Bd. VII., 1896, S. 134.

[3] For more detailed accounts of Kaiserling's method see Virchow's Archiv, Bd. 147, 1897, and Verhandl. d. deutsch. path. Gesellsch. München, 1899.

Methods especially suited for the demonstration of nuclear figures are the following:

(*α*) **Flemming's Method.**—Harden in Flemming's solution (see page 26), cut with the freezing microtome, embed in paraffine, stain with safranin, decolorize with acid alcohol (several seconds to half an hour), dehydrate in absolute alcohol until no further coloring matter is removed, clear in xylol, and mount in balsam. The resting nuclei are pale or colorless, those in the various stages of karyokinesis are stained intensely red. Instead of employing safranin, the osmic acid may be removed from the section by treatment with hydrogen peroxide, when Heidenhain's or Mallory's iron hæmatoxylin stain may be employed.

(*β*) **Heidenhain's Iron Hæmatoxylin.**—This and the following stain bring out the finer nuclear structures, the centrosomes, etc. Solutions required are:

I. Mordanting and Differentiating Solution:

Ferric ammonium sulphate,	2½ to 4 grams.
Water,	100 "

II. Staining Solution: ½-per-cent. aqueous hæmatoxylin solution.

Method.—1. The section (embed in paraffine, fasten to slides and remove paraffine with xylol) is placed for two hours in solution I.

2. Thoroughly wash in distilled water.

3. Transfer to solution II. for from one half to several hours.

4. Wash thoroughly in running water.

5. Differentiate in solution I. until the section becomes grayish blue or grayish black (control under the microscope; nuclear structures must be deep blue, protoplasm colorless). The longer the mordanting and staining process is continued the more definite become the centrosomes and the achromatic spindles.

6. Wash in water, alcohol, xylol, balsam.

(*γ*) **Mallory's Iron Hæmatoxylin.**

I. Mordanting and differentiating fluid tincture ferrichloride U. S. P.

II. Staining solution. Dissolve a few crystals of hæmatoxylin in a moderate amount of distilled water, heating over a Bunsen burner.

Method.—1. The section is placed for a few minutes in solution I.

2. Wash in water.

3. Place in solution II. for one to two minutes.

4. Differentiate in dilute solution of No. I. (made by adding a few drops of solution I. to a small dish of water; proper concentration is readily obtained by experiment), until proper differentiation is obtained.

5. Rinse in water, alcohol, xylol, balsam.

Celloidin sections may be treated in the same manner with the introduction of 93-per-cent. alcohol and carbol-xylol. Either of the iron hæmatoxylin stains may be employed with material hardened with any of the ordinary fixatives.

(*b*) **Protoplasmatic Structures.** (*α*) **Altmann's Granula Stain.**—Fresh fragments of tissue are hardened in

Potassium bichromate 5-per-cent. aqueous solution,	āā 24 hours.
Osmic acid, 2-per-cent. aqueous solution,	

Wash thoroughly in running water; treat with increasing alcohols; embed in paraffine; thinnest possible sections; fasten to slides; remove paraffine.

Staining solution I.

Aniline water, (See Weigert's fibrin stain.)	100 cc.
Acid fuchsin,	20 g.

Staining solution II.

Concentrated alcoholic picric acid solution,	50 cc.
Water,	100 "

Method.—1. Pour a few drops of solution I. over the object upon the slide. Heat over the flame until steam is given off.

2. When cold wash with solution II.

3. Again pour solution II. on the slide and warm slightly for a few seconds.

4. Drain off fluid, treating with absolute alcohol, xylol, balsam.

The length of time the preparation is treated with picric acid must be determined for each specimen.

The granulæ of the protoplasm are stained red, nuclei are colorless.

(β) **Beneke's (Kromayer's) Modification of Weigert's Fibrin Method.**—Harden in alcohol.

Method.—1. Stain ten to twenty minutes with aniline water gentian violet. (10 parts aniline oil and 100 parts of water are vigorously shaken together and filtered through a moist filter paper. After filtration 5 to 10 parts concentrated alcoholic solution of gentian violet are added.)

2. Wash in water.

3. Place in dilute Lugol's solution (color of port wine) one minute.

4. Carefully blot the section upon the slide with filter paper.

5. Differentiate in a mixture of two parts of aniline oil and three parts of xylol. The differentiation of the various structures stained by this method must be controlled under the microscope. They are the protoplasmatic elongations of the epithelial cells of the skin, connective tissue fibers, Sharpey's fibers in bone, neuroglia.

6. Thorough washing in xylol. Mount in balsam.

(γ) **The Horny Structures of the Skin. Gram's Method** (see page 64). (Ernst.) The hornifying and hornified cells are filled with deep blue granules (keratine granules) or take on a diffused blue color. Harden in alcohol.

2. Fibrin Stains. (*a*) **With Picro-carmine.**—In sections stained with picro-carmine, the fibrin takes on a brilliant yellow coloration.

(*b*) **With Hæmatoxylin Eosin or Methylene-blue Eosin.**—With either of these stains the fibrin takes on a red color.

(*c*) **With Weigert's Fibrin Stain.**—The following reagents are required for this stain.

1. Aniline water gentian violet made by shaking together 100 cc. of water and 10 cc. aniline oil. After thorough shaking, when the drops of oil are as finely divided as possible, the mixture is filtered through a wet filter paper. To the clear filtrate is added 10 cc. of concentrated alcoholic gentian violet solution. The mixture must be filtered each time before use and does not keep longer than a few weeks. (For permanent solutions of aniline water gentian violet see Lubarsch's method for demonstration of glycogen.)

2. Lugol's solution of iodine in potassium iodide:

Iodine,	1 part.
Potassium iodide,	2 parts.
Water,	300 parts.

3. Aniline oil-xylol :

Aniline oil,	2 parts.
Xylol,	1 part.

This method gives the best result with material which has been hardened in alcohol or formaline, when the hardening has not been continued too long. Material which has been hardened in Müller-formaline or Müller's fluid or too long in formaline does not give good results. Where it is absolutely necessary to employ material which has been hardened with the last named agents, the staining qualities of the sections may be improved by placing them for a few hours in a 5-per-cent. oxalic acid solution. Even in this case the action of the oxalic acid solution is not always sufficient to insure a proper reaction of the tissue to the stain. The sections must be cut on the freezing microtome or embedded in paraffine. Celloidin stains deeply with aniline dyes, which are removed with difficulty. The sections are best stained with alum or lithium carmine and after washing are placed in aniline water gentian violet solution for from five to ten minutes. Wash in water, transfer to Lugol's solution one to two minutes. As soon as the treatment with iodine solution is completed (the time must be observed with a watch) the section is drawn out upon a slide, carefully spread out and thoroughly blotted with a filter paper, pressing it firmly upon the glass to insure the removal of all superfluous water. The section and slide are then immediately transferred to a dish of aniline oil xylol, where the process of decolorization and differentiation takes place. The dish containing the slide immersed in the aniline oil-xylol mixture is rocked from side to side and as the color comes off fresh mixture is applied. The decolorization is continued out until the portions of the tissue which give up the blue color begin to appear red ; in other words, until the nuclear stain begins to make its appearance in the clear portions of the section, when the differentiation is complete. The final test as to whether the tissue is properly decolorized must be made with the microscope. When, as occasionally occurs, it is considered desirable to carry out the Weigert fibrin method on celloidin sections, the excess of coloring matter in the celloidin section may be removed according to Saxer, by treatment, after differentiation, with pure aniline oil in which a sufficient number of iodine crystals have been dissolved to give it a deep brown color. In either case, when the sections are properly differentiated, they are washed in pure xylol, the excess removed with a filter paper and the section embedded in Canada balsam. If the aniline oil is not properly removed by thorough treatment with xylol, the stain gradually fades. When the section is properly differentiated the nuclei are red, fibrin and various bacteria deep blue ; connective tissue, mucus, horny epidermis and elastic fibers (especially in material hardened in formaline) take on a blue color. The reaction of the various elements is rather uncertain, for which reason it is impossible to determine by the color, exactly what the nature of a structure in the preparation may be.

(*d*) **With Kochel's Method.**—The following solutions are required for this method :

I. 1- to 5-per-cent. aqueous solution chromic acid.

II. Weigert's hæmatoxylin solution—

Hæmatoxylin,	1
Absolute alcohol,	10
Aqua dest.,	90

(When the above ingredients are dissolved add concentrated watery solution of lithium carbonate, 1 cc.)

III. Aqueous solution of alum 10 per cent.

IV. Weigert's borax ferrocyanide of potash solution—

Borax,	2.
Ferrocyanide of potash,	2.5
Aqua dest.,	100.

This method is suitable for material which has been hardened in any of the ordinary fixatives and is best employed where Weigert's method is not applicable. Sections of tissue hardened with Kaiserling's method for the preservation of natural colors, sublimate or formaline, must be treated for a few moments with a 5-per-cent. acetic acid solution before placing in the chromic acid solution. This treatment assists in the decolorization of the red blood corpuscles in the tissue. The steps of the procedure are as follows:

1. Embed in paraffine.
2. Thinnest possible sections, which should be fastened to the slide with albumin-glycerine.
3. Place the deparaffinized sections for 5 to 10 minutes in solution I. (Chromic acid.)
4. Short washing in white porcelain dish (5 to 20 minutes) until the preparations become pale but still retain a definite yellow color.
5. Stain for 15 to 20 minutes in Weigert's hæmatoxylin (solution II.).
6. Wash in water.
7. Place for one minute in concentrated aqueous solution of alum until the sections take on a deep blue color.
8. Wash in water.
9. Differentiate in Weigert's borax-potassium-ferrocyanide solution (solution IV.) diluted with 3 parts water, 3 to 6 minutes.
10. Wash in water.
11. Place in concentrated aqueous solution of alum (solution III.) for from 15 minutes to 1 hour.
12. Wash in water.
13. Counter stain with alum carmine or safranin.
14. Dehydrate in alcohol, xylol, Canada balsam.

The fibrin is stained a blue black or deep blue; groundwork of the section is pale or colorless.

3. **Amyloid.**—The various reactions for amyloid material give the best results with fresh sections. Inferior results may be obtained with sections from material hardened in alcohol, sublimate, formaline, or Müller's fluid. An exception to the rule is Mallory's method for the demonstration of connective tissue, for which the material must be hardened in Zenker's fluid.

(*a*) **Iodine Reaction.**—Sections are placed in dilute Lugol's solution where they are allowed to remain for a few minutes and are then thoroughly washed. Clear and embed in glycerine. Amyloid is brownish red, the remaining structures yellow. The sections treated with iodine cannot be preserved, as the color rapidly fades.

(*b*) **Iodine Sulphuric Acid Reaction.**—The sections are treated with Lugol's solution as in the above method and after washing are placed in a 1-per-cent. aqueous solution of sulphuric acid, washed in water and embedded in glycerine. The structures which contain amyloid take on a blue-green or violet color; the remaining portions of the tissue are unchanged. This reaction cannot always be obtained.

Certain substances besides amyloid give a characteristic reaction when treated with iodine. (*a*) Starch takes on a blue color. (*b*) Certain bacteria, especially those found in the buccal cavity, likewise stain a blue color. (*c*) Cellulose, which gives with iodine a yellow color which is changed to blue when treated with sulphuric acid. (*d*) So-called corpora amylacea; round or angular bodies found in the prostate and central nervous system which stain either brown or blue with iodine, and when treated with iodine and sulphuric acid give either a blue or occasionally a red color; with aniline colors they give a reaction similar to amyloid. (*e*) Cholesterin crystals; when treated with iodine these remain colorless, but when afterward exposed to the action of sulphuric acid the edges of the crystals take on a deep-blue color.

(*c*) **Methyl-violet Reaction.**—1. Stain the section in a 1-per-cent. aqueous solution of methyl-violet (a few minutes).

2. Rinse in water to which has been added a few drops of glacial acetic acid.
3. Rinse in fresh water.
4. Embed in potassium acetate solution.

Amyloid is deep red, the nuclei dark blue, the remainder of the tissue greenish-blue. The differences in color reaction are most definite when examined with light which is reflected from the clouds. With the blue light of the sky the reaction is not so well defined.

(*d*) **Methyl-green.**—The steps in this method are the same as those given under methyl-violet, except that a 1-per-cent. solution of methyl-green is substituted for the solution of methyl-violet.

Amyloid stains violet, nuclei and remaining tissue green. The differences in color reaction may be readily observed when examined with artificial light, which should be employed where this is necessary. Embed in potassium acetate.

The reaction which amyloid material gives with the aniline dyes becomes more definite and sharply defined, as the solution of the stain is diluted. For this reason the most definite results are obtained by using a very dilute solution of the stain, allowing the sections to remain in it for a longer period of time.

(*e*) **Polychrome Methylene-blue.** (After Schmorl.)—1. Stain with polychrome methylene blue 10 to 15 minutes.

2. Wash in water.
3. Place for 10 to 20 seconds in a one half of 1-per-cent. solution of acetic acid.
4. Transfer to a solution of alum (concentrated solution of alum 1 part, water 1 part), 2 to 5 minutes.
5. Rinse in absolute alcohol one half minute.
6. Dehydrate in fresh absolute alcohol, one quarter to one minute.
7. Clear in xylol, mount in balsam.

Amyloid material is stained pale red, nuclei deep blue, protoplasm light blue. This reaction remains for a considerable period of time, especially when the preparation is kept in the dark.

(*f*) **Mallory's Connective Tissue Stain.**—With this method the deposits of amyloid in the tissue, especially in the liver, stand out clearly, taking on a light blue color, by which the amyloid may be differentiated from the deep blue connective tissue fibrils and the bright red protoplasm of the liver cells.

4. **Glycogen.**—The amount of glycogen in the tissue may be roughly determined by examining scrapings or sections which have been treated with Lugol's solution when the glycogen may be determined by the brown color given it by the iodine. The glycogen rapidly dissolves in water so that preparations thus made cannot be preserved. To preserve the glycogen in the

tissue it must be hardened immediately after the operation at which the material is obtained, or directly after death when taken from the cadaver, by placing in absolute alcohol. Caution is necessary as glycogen is rapidly disintegrated in the cadaver and dissolved by all aqueous fixing fluids. Glycogen is best stained with

(*a*) **Iodine.** (*α*) **Langhans' Method.**—1. Stain in Lugol's solution five to fifteen minutes.

2. Dehydrate in a mixture of absolute alcohol four parts, officinal tincture of iodine one part.

3. Clear and examine in origanum oil.

Lubarsch recommends a previous staining of the section with acid alcohol carmine one half to one minute. Differentiate in acid alcohol, rinse in absolute alcohol and stain according to Langhans. When the preparation is embedded in origanum oil, too rapid evaporation of the oil may be prevented by sealing the cover-glass with paraffine or sealing wax. Such preparations may be preserved for a period of six months or longer.

(*β*) **Simple Iodine Stain.**—1. Stain section with hæmatoxylin.

2. Rapidly wash in water.

3. Transfer to Lugol's solution (several minutes).

4. Transfer to absolute alcohol and wash until the section begins to take on a bluish color.

5. Xylol. Canada balsam.

(*b*) **Method of Lubarsch.**—1. Stain the section in acid alcohol carmine.

2. Stain in concentrated aniline water gentian violet solution (two to four minutes).

For the preparation of the above concentrated aniline water gentian violet solution the following two solutions should be kept, and as they do not deteriorate, may be used indefinitely:

Solution I.:

Absolute alcohol,	33
Aniline oil,	9
Gentian violet in excess.	

Solution II.: Concentrated aqueous solution of gentian violet. Before use mix three parts of solution I. to 17 parts of solution II. The older the solutions become the better they are suited for this method.

3. Rapid washing in water.

4. Short treatment with Gram's iodine solution (about 15 seconds).

5. Rapid drying of the section with filter paper.

6. Decolorize in aniline oil-xylol (2 : 1).

7. Thorough removal of the aniline oil with xylol.

8. Canada balsam.

The drops of glycogen in the tissue are stained deep blue or violet.

5. **Mucus.**—Mucus is usually stained in hæmatoxylin preparations when it takes on a blue, occasionally a reddish coloration, dependent upon the method of hardening. The various forms of mucus found in the body stain differently.

(*a*) **Weigert's Fibrin Stain.**—Mucus is stained blue violet.

(*b*) **Methylene-blue.**—Mucus takes on a dark blue color.

(*c*) **Mallory's Connective Tissue Stain.**—The color of mucus with this method is somewhat variable, but usually takes on a pale sky-blue.

(*d*) **Polychrome Methylene-blue.** (Unna.)—1. Tissue is hardened in alcohol and embedded in paraffine or celloidin.

2. Stain with polychrome methylene-blue 5 to 10 minutes.[1]

3. Wash the sections in a dish of water to which has been added a few drops of acetic acid.

4. Rapid dehydration in absolute alcohol.

5. Clear in xylol, mount in balsam.

Mucus is stained rose red.

(*e*) **Thionin.** (Hoyer.)—1. Thin pieces of tissue are hardened in 5-per-cent. aqueous solution of corrosive sublimate (a few hours).

2. Wash in 80-per-cent. alcohol and reharden in absolute alcohol.

3. Embed in paraffine.

4. Place the section for thirty seconds in a concentrated aqueous sublimate solution.

5. Wash in alcohol.

6. Stain in dilute aqueous solution of thionin (2 drops of a saturated aqueous solution to 5 cc. of water) 5 to 15 minutes.[2]

7. Wash in 90-per-cent. alcohol.

8. Rapid dehydration in absolute alcohol, xylol, balsam.

The mucus takes on an intense red color, which is more apparent when the sections are embedded in glycerine.

Material which has been hardened in alcohol may be made suitable for this reaction by placing from one half to one minute in concentrated aqueous sublimate solution before staining.

6. **Elastic Fibers.** (*a*) **With Acid Orcein, According to Unna-Tænzer.**—The staining solution is prepared as follows:

Orcein,	.5 gram.
Aqua dest.,	20. cc.
Alcohol,	50. cc.
Hydrochloric acid,	20. drops.

Old solutions give the best results.[3]

Material which has been hardened in alcohol, formaline, or Müller-formaline is suitable. Embed in paraffine or cut with the freezing microtome.

1. Sections are immersed in a dish of orcein solution and placed in a thermostat for five minutes or kept at room temperature for half an hour. If the stain is to be placed in a thermostat, the dish or bottle must be closed with a stopper to prevent evaporation of the alcohol.

2. Wash sections in 60-per-cent. alcohol.

3. Dehydrate in absolute alcohol.

4. Clear in xylol, mount in balsam.

Where the sections have been overstained the color may be removed by treating them with acid alcohol followed by washing in 60-per-cent. alcohol. Sections may be first stained with alum or lithium carmine before staining with orcein or may be given a counter stain with methylene-blue or thionin afterward.

The elastic fibers are stained brown or brownish black with this method.

As the necessity of employing material which has already been embedded in celloidin occasionally arises, the following method of Laurent for the removal of the orcein from the celloidin may be useful: After the differentiation in acid alcohol the preparation is transferred to a dish of water, to which has been added a moderate amount of liquor ammonii caustici. In this solution the section turns blue and liberates part of the coloring matter. When color fails to come from the section it is returned to acid alcohol, and if the celloidin is still found to be too darkly stained the operation may be repeated until the section is sufficiently decolorized. This procedure, according to Schieferdecker, does not injure the differentiation of the elastic fibers.

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[1] Polychrome methylene-blue solution may be obtained ready for use from Gruebler.

[2] Great care must be taken to see that the thionin is genuine. That obtained from Gruebler is reliable.

[3] Or the solution ready for use may be obtained from Gruebler.

(*b*) **With Weigert's Elastica Stain.**—Material which has been hardened in any of the usual fixatives may be employed. The sections may be cut on the freezing microtome, or from material embedded in paraffine or celloidin. The solution in which the sections are stained is prepared as follows:

Fuchsin (not acid fuchsin),	2 grams.
Resorcin,	4 "
Water,	200 cc.

These ingredients are placed in a dish and brought to the boiling point, when 25 cc. of liquor ferri sesquichlorati Ph.G.III is added and the whole is allowed to cool and is then filtered. The precipitate is allowed to dry on the filter paper and is then returned to the dish in which the mixture was first cooked. Two hundred cc. of 93-per-cent. alcohol is poured into the dish, placed over the water-bath, brought to the boiling point and constantly stirred. When all of the coloring matter is dissolved the filter paper is fished out, the solution is allowed to cool, is filtered and brought back to the volume of 200 cc. by the addition of 93-per-cent. alcohol. Four cc. of hydrochloric acid is then added to the mixture which is ready for use.

METHOD.—1. Sections are first stained with lithium carmine.
2. Place in Weigert's solution as above, 10 to 20 minutes.
3. Wash in dilute alcohol.
4. Dehydrate in absolute alcohol.
5. Clear in xylol. Mount in balsam.

When the section has been overstained it may be decolorized with acid alcohol. Elastic fibers are stained blue. This stain may be satisfactorily combined with van Gieson's stain, in the following manner:

1. Stain with hæmatoxylin.
2. Stain according to Weigert.
3. Counter-stain with picric acid fuchsin mixture (see p. 42).

The elastic fibers are blue, connective tissue red, fibrin yellow.

7. White Fibrous Connective Tissue and Reticulum. (*a*) **Mallory's Connective Tissue Stain.**—This method gives the best results with material which has been hardened in Zenker's fluid. Fair results may be obtained after hardening with Flemming's solution. This method does not differentiate connective tissue from reticulum.

The necessary solutions are:

I. 0.05- to 0.1-per-cent. solution of acid fuchsin.

II. 1-per-cent. aqueous solution of phosphomolybdic acid.

III. Aniline blue soluble in water (Gruebler),	0.5
Orange G (Gruebler),	2.0
Oxalic acid,	2.0
Water,	100.0

METHOD.—1. Fix in Zenker's fluid.
2. Embed in celloidin or paraffine.
3. Stain the sections in 1/20 to 1/10 of 1-per-cent. aqueous solution of acid fuchsin (solution I.) 1 to 3 minutes.

4. Wash in water.
5. Place in a 1-per-cent. aqueous solution of phosphomolybdic acid (solution II.) for one minute or longer.
6. Wash in two changes of water.
7. Stain in solution III. for from 10 to 20 minutes or longer.
8. Wash in water.
9. Dehydrate in 95-per-cent. alcohol.
10. Blot on the slide and clear in origanum oil.
11. Xylol, balsam.

Reticulum and fibrillæ of connective tissue stain a deep blue. Beside these, amyloid, mucus and certain hyaline substances take the blue stain but they may be recognized either by their variation in color or morphology. Nuclei, protoplasm, elastic fibers, axis cylinders, neuroglia fibers and fibrin stain red; red blood corpuscles and myelin sheaths yellow. Where it is desired to bring out the connective tissue as sharply as possible, omit the stain with acid fuchsin, in which case the nuclei and protoplasm stain yellow and the blue connective tissue fibrils stand out more prominently.

8. Fat. (*a*) **According to Flemming.** METHOD.—1. Harden with Flemming's or Hermann's solution.

2. Wash in water.
3. Transfer to formaline solution and cut with the freezing microtome, or,
3. Embed in paraffine.
4. Overstain with safranin 1- to 2-per-cent. aqueous solution.
5. Decolorize in acid alcohol.
6. Dehydrate in absolute alcohol.
7. Clear in origanum oil, mount in balsam.

The fat is turned a deep black by the action of the osmic acid. Nuclear structures are stained red with safranin.

(*b*) **According to Marchi.** METHOD.—1. Harden the tissue in Müller's fluid (one week).

2. Transfer to a solution of

Müller's fluid,	2 parts,
Osmic acid aqueous solution 1-per-cent.,	1 part,

in which the tissue remains seven days.

3. Transfer to 4-per-cent. formaline solution, and cut with the freezing microtome, or reharden in alcohol and embed in paraffine or celloidin.
4. Stain with hæmatoxylin.

Sections must be imbedded in glycerine or melted Canada balsam. Canada balsam which has been thinned with xylol dissolves the fat.

REMARK.—Marchi's method does not give reliable results with fœtal tissues.

(*c*) **With Sudan III.** (Daddi.)—This stain gives the best reaction with fresh sections (double knife or frozen sections) or with material which has been hardened in formaline and cut on the freezing microtome.

METHOD.—1. Sections cut on the freezing microtome are placed in a saturated 80-per-cent. alcoholic solution of Sudan III., for from 10 to 15 minutes.

2. Wash in 50-per-cent. alcohol for a half minute.
3. Wash in distilled water.
4. Stain in alum hæmatoxylin.
5. Embed and examine in glycerine.

The fat globules take on a bright orange red.

9. Demonstration of Iron in Pigment.—Pigments which contain iron are derived from the coloring matter of the blood. Where the deposit of pigment is old, the presence of the iron may no longer be demonstrable. The other forms of pigment found in the tissue do not contain iron. Fresh material and material which has been hardened in alcohol are suitable for the application of the various methods for the demonstration of iron. Formaline hardening is unsuitable. Sections may be cut on the freezing microtome or embedded in paraffine. In the manipulation of the sections glass rods which have been drawn out to fine points should be employed in place of the usual steel preparation needles.

(*a*) **Method of Perl's.**—1. Sections are stained with alum or lithium carmine.

2. Immerse for several minutes in a 2-per-cent. aqueous solution of potassium ferrocyanide.

3. Wash in acid alcohol.

4. Wash in water.

5. Mount in glycerine.

Permanent preparations may be made by transferring from the acid alcohol into absolute alcohol, xylol and balsam. Pigment which contains iron is stained blue.

(*b*) **Ammonium Sulphate Method.** (Quincke.)—1. Sections are first stained with alum carmine.

2. Immerse in a freshly prepared solution of ammonium sulphate until they take on a dark or greenish black coloration (5 to 20 minutes).

3. Wash in water.

4. Examine in glycerine, or dehydrate in alcohol, clear in xylol, mount in balsam.

A discoloration similar to that produced by the ammonium sulphate on the iron results when the tissue contains deposits of silver, lead or mercury. The pigment (iron-containing) takes on a greenish brown color.

(*c*) **With Ordinary Methylene-blue Stain.**—Pigment which contains iron is not stained with methylene-blue, whereas the other forms of pigment take on a bluish green color.

10. Blood.—See chapter on blood.

11. Plasma Cells.—These elements are demonstrated by the method of Unna, modified by Jadassohn and Marschalko.

Material which has been hardened in alcohol, or sublimate should be employed. Embed in paraffine.

METHOD.—1. Sections are stained with polychrome methylene-blue or dilute aqueous solution of thionin (one half to one hour).

2. Wash in water.

3. Decolorize in Unna's glycerine-ether mixture,[1] until the sections become gray blue (several minutes to hours); or decolorize in 70-per-cent. alcohol.

4. Thorough washing in water.

5. Dehydrate in absolute alcohol.

6. Clear in xylol. Mount in balsam.

The protoplasm of the plasma cells is stained deep blue. The protoplasm of the mast-cells contains bright red, spherical granules.

12. Bone.—Bone is best hardened with formaline or Müller-formaline and decalcified with sulphurous acid. The following stains are especially useful:

(*a*) **Van Gieson's Stain** (see page 42).—With this method cartilage is stained blue, osteoid substance red, bone yellow, calcified portions deep blue.

(*b*) **Schmorl's Method for the Demonstration of the Bone Corpuscles.**—Material which has been hardened in formaline, Müller-formaline, or Müller's fluid may be used. Decalcify with sulphurous acid or according to Ebner. Embed in celloidin.

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[1] Must be obtained from Gruebler.

I. Thionin solution.

This is prepared by diluting 2 cc. of a concentrated thionin solution in 50-per-cent. alcohol, with 10 cc. of water (the thionin must be genuine and pure [Gruebler] otherwise the reaction fails).

II. Saturated aqueous solution of picric acid.

METHOD.—1. Celloidin sections, after thorough washing in water (10 minutes), are stained in the above thionin solution 5 to 10 minutes or longer.

2. Wash in water.

3. Place for one half to one minute in saturated aqueous solution of picric acid.

4. Wash in water.

5. Differentiate in 70-per-cent. alcohol until no further color comes off (5 to 10 minutes).

6. Dehydrate in 95-per-cent. alcohol.

7. Clear in origanum oil.

8. Mount in balsam.

By the addition of one to two drops of liquor ammonii caustici to 10 cc. coloring solution, the prolongations of the bone corpuscles into the calcified portions of the trabeculæ may be stained; otherwise only the prolongations of the bone corpuscles in the osteoid tissues are demonstrated.

The bone corpuscles and their prolongations are stained a brilliant blue. The ground work of the trabeculæ pale blue.

13. Central Nervous System. (*a*) **Methods for the Demonstration of the Myelin Sheaths.** (*a*) **Weigert's Method, Modified by Pal.**

Tissue hardened in Müller's fluid or Müller-formaline is suitable. Freezing microtome sections or celloidin sections should be employed. Where the material has been hardened in formaline alone, it is best to reharden it in the following fluid:

Potassium or ammonium bichromate,	5 grams	Dissolve by heat and filter.
Chrome alum,	2 grams	
Aqua dest.,	100 grams	

Tissue remains in this fixative for from 4 to 5 days, at most 8 days, after which it is thoroughly washed in running water, rehardened in alcohol and embedded in celloidin. Too long a period in alcohol decreases the rapidity of staining, in which case the tissue must be placed for a period of time in a 1-per-cent. solution of chrome alum or in Müller's fluid. The solutions necessary for this method are:

I.	Hæmatoxylin,	1 part.
	Absolute alcohol,	10 parts.
	Aqua dest.,	90 parts.

II. Concentrated aqueous solution of lithium carbonate.

III. 0.25-per-cent. aqueous solution of potassium permanganate.

IV.	Oxalic acid pure,	1.0
	Potassium or sodium sulphate,	1.0
	Aqua dest.,	200.0

METHOD.—1. Stain the section for from 12 to 24 hours in the thermostat in

Weigert's hæmatoxylin solution (solution I.) to which has been added a few drops of saturated aqueous solution of lithium carbonate.

2. Wash in water, to which has been added a few drops of lithium carbonate solution.

3. Transfer to potassium permanganate solution (solution III.) one half to one minute.

4. Wash in water.

5. Differentiate in oxalic acid mixture (solution IV.).

This requires several minutes and must be continued until the brownish color has disappeared, and the portions of the tissue which contain axis cylinders have taken on a bluish black. When the differentiation proceeds too slowly or does not develop, the sections must be returned to the permanganate solution.

6. Thoroughly wash in water. (When desired counterstain with alum carmine.)

7. Dehydrate in 93-per-cent. alcohol.

8. Carbol-xylol, balsam.

(β) **Bolton's Stain for the Myelin Sheaths.**—This method is suitable for material hardened in formaline or Müller-formaline. Solutions employed are:

I. 1-per-cent. aqueous solution of osmic acid.

II. Acetic acid hæmatoxylin.

Hæmatoxylin,	1 gram.
Glacial acetic acid,	2 cc.
Water,	100 cc.

Method.—1. Frozen sections or celloidin sections are placed for several minutes in 1-per-cent. osmic acid solution.

2. Wash in water.

3. Stain with acetic acid hæmatoxylin for several hours at room temperature or for two hours in the thermostat.

From this point the section is manipulated according to Weigert-Pal beginning at step 2 of that method.

(b) **Methods for the Demonstration of Axis Cylinders.** (α) **Van Gieson's Method.**—Suitable for material which has been hardened in Müller-formaline or formaline. Freezing microtome sections or embed in celloidin. Axis cylinders are brownish red, neuroglia yellow, connective tissue red.

(β) **Nigrosin.**—Harden in Müller-formaline or Müller's fluid. Freezing microtome sections or embed in celloidin.

Method.—1. Stain the sections in a thin aqueous solution of nigrosin (15 to 30 minutes).

2. Wash in water.

3. Dehydrate in 93-per-cent. alcohol.

4. Carbol-xylol, balsam.

The axis cylinders and neuroglia are stained a gray blue color.

(γ) **Ammonium Carmine.**—This carmine solution is prepared as follows:

Carmine,	1 g.
Liquor ammonium caustici,	1 cc.
Aqua dest.,	100 "

The mixture must be allowed to ripen by placing it for 24 hours in an open flask, after which it is filtered and again allowed to stand in an open vessel for several weeks. METHOD.—Harden in formaline or Müller-formaline. Freezing microtome or celloidin sections.

1. Stain in very dilute (pale red color) ammonium carmine 12 to 24 hours.
2. Wash in water.
3. Dehydrate in absolute alcohol.
4. Xylol, balsam.

When thought desirable the sections may be first stained with hæmatoxylin. The axis cylinders are stained red.

(*c*) **Method for the Demonstration of Nissl's Bodies in the Protoplasm of Ganglion Cells.** (Juliusburger and Meyer.)—Harden in Müller-formaline solution for four days in the cold. Freezing microtome sections or embed in celloidin.

METHOD.—1. Sections are stained in a one-per-cent. aqueous solution of thionin for from one half to one minute (warm during the staining process).

2. Decolorize in 95-per-cent. alcohol, until sufficiently differentiated. Control under the microscope in bergamot oil.

3. Carbol-xylol, balsam.

Nissl's bodies are stained bright blue.

(*d*) **Neuroglia Stain.**—Only material which has been removed from the cadaver within 12 hours after death is suitable for any of the following methods: None of them gives results with animal tissues.

(*α*) **Mallory's Method for Neuroglia.**—1. Thin pieces of tissue are hardened in 4-per-cent. formaline 4 days in a thermostat.

2. Transfer to saturated aqueous solution of picric acid 4 to 8 days in the thermostat.

3. Transfer to 5-per-cent. aqueous solution of ammonium bicarbonate, 4 to 7 days in the thermostat.

4. Reharden in alcohol without washing.

5. Embed in celloidin.

6. Stain according to Weigert's fibrin method. Decolorize with aniline oil-xylol until proper differentiation is reached.

7. Rinse in xylol.

8. Mount in balsam.

The neuroglia fibers are stained a deep blue. The method may be shortened by removing the tissue from the ammonium bichromate solution (No. 3) and placing it in 10-per-cent. formaline, after which it may be cut with the freezing microtome.

(*β*) **Mallory's Connective Tissue Stain Modified for Neuroglia.** HARDENING METHOD.—1. Fix thin pieces of nerve tissue in 4-per-cent. aqueous solution of formicaldehyde (10-per-cent. formaline) for at least four days in the thermostat.

2. Saturated aqueous solution of picric acid four days or more.

3. Five-per-cent. aqueous solution of bichromate of ammonium 4 days in the thermostat (use an abundance of the solution and change at the end of 24 hours).

4. Reharden in alcohol.

5. Embed in celloidin.

STAINING METHOD.—1. Sections are stained in a 1-per-cent. aqueous solution of acid fuchsin.

2. Wash quickly in water.

3. One-per-cent. aqueous solution of phosphomolybdic acid 1 to 2 minutes.

4. Wash in two changes of water.

5. Three to 5 minutes in the aniline blue and orange G solution. (See page 54.)

6. Wash in water.

7. Dehydrate in 95-per-cent. alcohol.
8. Blot and clear with xylol or oleum origani cretici.
9. Xylol, balsam.

Connective tissue blue, neuroglia fibers deep red, axis cylinders and ganglion cells a lighter red.

(γ) **Stain for Neuroglia Cells, with the Prolongations and Axis Cylinders.** (Mallory.)—Tissue is hardened in Müller's fluid or Müller-formaline. The staining solution is composed of:

Hæmatoxylin,	1.75 g.
0.5-per-cent. aqueous solution of phosphomolybdic acid,	200 g.
Carbolic acid crystals,	5 g.

The solution must remain for two or three weeks in sunlight until it takes on a deep violet color.

METHOD.—1. Stain celloidin sections 5 minutes to 1 hour in the above solution.
2. Wash in water.
3. Decolorize in 50-per-cent. alcohol.
4. Dehydrate in 93-per-cent. alcohol.
5. Origanum oil, balsam.

The neuroglia cells and their prolongations are stained a deep blue. The axis cylinders are likewise stained blue.

(e) **Simplified Golgi's Method for the Demonstration of Ganglion and Neuroglia Cells.** METHOD.—1. Harden in 10-per-cent. formaline, or Müller-formaline solution 24 hours or longer.
2. Transfer thin slices of the material (less than 1 mm. in thickness) in 1-per-cent. silver nitrate solution. Place in the dark for 24 hours.
3. Rapid washing in water.
4. Place in 10-per-cent. formaline solution for several hours and cut with the freezing microtome.
5. Dehydrate in absolute alcohol.
6. Xylol, balsam.

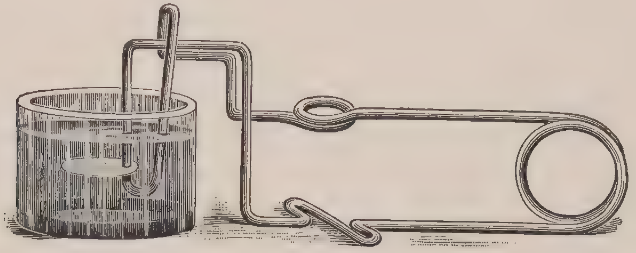
The ganglion cells and neuroglia cells are stained an intense black. The sections are not permanent.

REMARK.—All of the above methods may be carried out with material which has been hardened in 10-per-cent. formaline. The various steps of re-hardening, mordanting, etc., may be applied to slices of tissue or sections from the freezing microtome.

14. Staining Methods for the Demonstration of Bacteria. (a) **In Preparations made on Cover-Glasses or Slides.**—Cover-glass or slide preparations made according to the method given on page 28 by spreading fluid containing the organisms upon cover-glasses or slides, allowing them to dry and then passing them three times through the flame, are ready for staining.

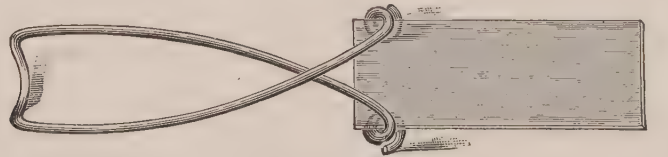
(α) **Simple Stains.** 1. METHYLENE-BLUE.—Methylene-blue is best kept in a concentrated alcoholic solution. A few drops of this stock solution added to a small dish of water affords a suitable dilution for staining purposes. Otherwise a 1- to 2-per-cent. aqueous solution of the stain may be prepared and used in full strength. In staining prepared cover-glasses these are placed upon the surface of the fluid with the prepared side downward, and allowed to float upon the surface. Five to ten minutes is usually sufficient and the staining process may be shortened by warming the fluid while the cover-glass is floating upon the surface. By using a suitable forceps (Fig. 7) the cover-slip may be immersed in the fluid. When the time required for staining has passed, the cover-slip is removed with a suitable forceps, rinsed in water and blotted between layers of filter paper. When the cover-slip is thoroughly dry, it is embedded in Canada balsam, taking care to turn the prepared surface downward. When the preparations are overstained they may be decolorized in water containing a few drops of acetic acid or in alcohol. Where the preparation is made upon a slide and not upon a cover-glass, the fluid may be poured upon the slide and washed off under a small stream of running water. A convenient device for holding the slides, during staining, is shown in Fig. 8. After blotting with filter paper, a few drops of immersion oil may be placed upon the surface of the slide and the cover-slip dispensed with, the examinations being made with the oil immersion brought into apposition with the drop of immersion fluid with which the stained material is covered. Cover-glasses may be stained in a similar manner by employing a cornet forceps, which holds the cover-slip firmly in place and is so arranged that the fluid cannot run upon the jaws of the instrument.

FIG. 7.



Kalteyer's forceps for immersing cover-slips during staining.

FIG. 8.



Kirkbride's slide holder for staining on the slide.

2. BISMARCK-BROWN.—This stain is employed in solution of the same strength and in the same manner as that described for methylene-blue.

3. LÖFFLER'S METHYLENE-BLUE, which is prepared by mixing concentrated

Alcoholic solution of methylene-blue,	30 parts.
0.01-per-cent. potassium hydrate solution,	100 parts.

This solution is useful for the demonstration of certain forms of bacteria which stain with difficulty. The method of application is the same as that of Nos. 1 and 2.

4. ANILINE WATER GENTIAN VIOLET.—(For method of preparation, see Fibrin Stain, page 48.) The application of the stain is the same as No. 1. In place of the usual aniline water gentian violet, Sterling's aniline gentian violet may be employed.

Saturated aqueous solution of gentian violet,	90 parts.
Aniline oil,	2 parts.
Alcohol, 93 per cent.,	8 parts.

The aniline oil and alcohol are thoroughly mixed and then added to the gentian violet solution. The whole is thoroughly shaken and filtered.

5. Carbol-fuchsin. (Ziehl's.)

Fuchsin,	1 part.
Alcohol,	10 parts.
5-per-cent. aqueous solution carbolic acid,	100 parts.

This stain is employed for the demonstration of tubercle bacilli.

Method.—1. Prepared cover-slips or slides are stained with the above solution for from 5 to 10 minutes. During the process of staining the slide or slip should be held over the flame and heated until steam begins to rise.

2. Wash in water.
3. Decolorize in acid alcohol, until the cover-glass or slide appears colorless.
4. Wash in water.
5. Counter-stain with methylene-blue.
6. Wash in water.
7. Blot between filter papers.
8. Embed in Canada balsam.

The tubercle bacilli which are not decolorized by the acid alcohol retain a bright red color. The remainder of the preparation stains blue.

(β) **Gram's Method.**—1. Prepared cover-slips or slides are stained with aniline water gentain violet or Sterling's gentian violet for from 3 to 5 minutes.

2. Rapidly wash in water.
3. Place in Lugol's solution one to one and one half minutes—

Iodine,	1.
Potassium iodine,	2.
Aqua dest.,	300.

4. Decolorize in absolute alcohol until the preparation appears nearly colorless.
5. Blot between filter papers.
6. Embed in Canada balsam.

Certain bacteria (staphylococci, streptococci, etc.) are stained a deep blue by this method, while the remainder of the preparation remains colorless. Bismarck-brown may be used as a counter-stain.

(γ) **Friedlander's Capsule Stain.**—Staining solution:

Concentrated alcoholic gentian violet solution,	50.
Water,	100.
Glacial acetic acid,	10.

Method.—1. Immerse the cover-glass in a 1-per-cent. solution of acetic acid 1 to 3 minutes.

2. Stain for half a minute in the above solution.
3. Differentiate in 1-per-cent. acetic acid solution.
4. Rinse and examine in water if the differentiation has been successful.
5. Dry between filter papers and embed in Canada balsam.

The above method may be applied to sections which must be stained for 24 hours, after which they are differentiated in 1-per-cent. acetic acid solution. Dehydrate in alcohol, xylol, balsam.

(δ) **Capsule Stain.** (Welch.) Method.—1. Cover the prepared surface of the slide or cover-slip with glacial acetic acid for a few seconds.

2. Drain off and replace with aniline water gentian violet.
3. Wash in 2-per-cent. sodium chloride solution.
4. Examine in sodium chloride or dry and mount in balsam.

(ε) **Stain for Spores.**—Spores in organisms may be demonstrated by over-staining with carbol-fuchsin, using heat, and decolorizing in acid alcohol. Counter-stain in methylene-blue. The spores are red, bacteria blue.

(ζ) **Stain for Flagella.**—Solutions required for this stain are as follows:

Solution I. (Mordanting solution).

20-per-cent. aqueous solution of tannin,	10 parts.
Cold saturated solution of ferrous sulphate,	5 cc.
Saturated alcoholic solution of fuchsin,	1 cc.

Solution II. Concentrated alcoholic solution of fuchsin.

Solution III. Silver oxide ammonia solution for Welcke's modification:

Nitrate of silver 4 grams are dissolved in 8 to 10 cc. aqua dest. To this are added a few drops of ammonia until a brown precipitate is formed. Ammonia is added drop by drop until the brown precipitate is dissolved. Add aqua dest. until the solution reaches 100 cc.

The flagella of bacteria are best demonstrated in organisms which have been taken from agar cultures one day old. Select a thoroughly clean cover-glass or slide. Spread the material well to isolate the organisms. Allow the material to thoroughly dry upon the slide or cover-slip.

METHOD.—1. A small amount of the culture is removed with a platinum needle, being careful that no agar is taken up. This is diluted in a watch-glass of warm water, after which a drop is carefully spread upon a clean cover-glass or slide.

2. Allow the preparation to dry thoroughly.

3. Fix by drawing rapidly through the flame.

4. Mordant with solution I. heating lightly over the flame.

5. Rinse in water and dry the preparation.

6. Stain with dilute alcoholic fuchsin solution. (1 cc. alcoholic fuchsin solution to 9 cc. water. Heat gently.)

7. Wash in water. Blot between filter papers.

8. Mount in Canada balsam.

A modification of the above method is recommended by Welcke. The steps from 1 to 5 are the same.

6. The cover-slip or slide is covered with silver oxide ammonia solution and heated until steam is driven off. When the portion of the slide containing the stained material becomes a distinct brown, 2 or 3 minutes, the cover-slip is washed in water and dried with filter paper (the silver oxide solution must not dry upon the cover-slip or a precipitate will be formed).

7. Place in a 1-per-cent. solution of corrosive sublimate one quarter of a minute.

8. Rinse in water. Dry with filter paper.

9. Repeat step 6. 1 to 3 minutes.

10. Wash in water. Dry with filter paper.

11. Place the slide in Rodinol 1 : 18 water, or ordinary metal developer one quarter of a minute.

12. Wash in water. Dry with filter paper.

Bacteria which are readily stained do not require the repetition of step 6. The disintegration of the flagella which occasionally occurs during the first drying of the cover-slip may be overcome by diluting the material from the culture with 10-per-cent. formaline solution or 1-per-cent. osmic acid solution instead of water.

(*b*) **Methods for the Demonstration of Bacteria in Sections.**—Material which has been hardened in alcohol, sublimate, formaline and Müller-formaline is suitable for any of the following methods. Sections may be cut with the freezing microtome or from material embedded in paraffine. Celloidin sections are not suitable.

(α) **With Methylene-blue or Löffler's Methylene-blue Solution.** METHOD.—1. Stain the sections in methylene-blue or Löffler's methylene-blue solution 5 to 15 minutes.

2. Rapidly wash in water.
3. Decolorize with acidulated water or 93-per-cent. alcohol.
4. Dehydrate in absolute alcohol.
5. Clear in xylol. Mount in balsam.

Certain forms of bacteria which are stained with difficulty, as, for instance, the bacillus of glanders, may be stained with Unna's plasma cell stain. (Polychrome methylene-blue.)

(β) **With Gram's Method.**—Sections are previously stained with alum carmine or lithium carmine.

1. Stain sections in aniline water gentian violet or Sterling's violet.
2. Wash in water.
3. Place in dilute Lugol's solution one to one and one-half minutes.
4. Decolorize in absolute alcohol until the carmine stain appears.
5. Clear in xylol. Mount in balsam.

Celloidin sections may be decolorized according to Saxer by placing them after treatment with dilute Lugol's solution, in aniline oil, to which have been added a sufficient number of iodine crystals to give it a dark brown color. In this reagent the celloidin is decolorized.

Certain bacteria, pus cocci, etc., are stained a deep blue with Gram's method, the nuclei of the tissue red. Similar results may be obtained with Weigert's fibrin method.

(γ) **With Carbol-fuchsin for the Demonstration of Tubercle Bacilli.**—1. The sections are stained in carbol-fuchsin solution, gently heating, for from 5 to 15 minutes. Or, the dish containing the stained sections may be placed for several hours in the thermostat at 36° C.

2. Wash in water.
3. Decolorize in acid alcohol, until the sections are colorless.
4. Wash in water.
5. Rapidly counter-stain with methylene-blue. (Overstaining must be avoided.)
6. Wash in water.
7. Absolute alcohol.
8. Xylol, balsam.

Sections may likewise be stained with hæmatoxylin, either before or after staining with fuchsin.

15. Methods for the Demonstration of Yeasts and Moulds.—Ordinary yeast organisms are best examined in the fresh state, but may be stained in cover-glass or slide preparations after the manner described for the demonstration of bacteria. They may be stained in sections according to Busse in the following manner:

1. Sections are first stained with alum hæmatoxylin 15 minutes.
2. Stain in dilute carbol-fuchsin solution (1 part of Ziehl's fuchsin solution to 20 parts of water), 30 minutes to 24 hours.
3. Decolorize 15 seconds to 1 minute in 95-per-cent. alcohol.
4. Absolute alcohol.
5. Xylol, balsam.

Nuclei are stained deep blue or violet, yeast cells pale red.

The conidiæ of the yeast organism are usually surrounded by a capsule.

Moulds are best examined fresh in 2- to 3-per-cent. potassium hydrate solution. A great many forms of moulds cannot be properly examined in water, in which case 60-per-cent. alcohol, to which a few drops of ammonia have been added, should be employed. Examine in glycerine. In hardened material the moulds and various forms of fungi react very uncertainly to the usual staining methods, for which reason all of the available methods should be employed. (Methylene-blue, Löffler's methylene-blue, Weigert's fibrin method, simple hæmatoxylin.)

Material is best hardened in Müller-formaline or formaline-alcohol solution. The fungus of thrush or aphthæ is readily stained with Gram's or Weigert's fibrin method.

16. Mallory's Method for the Differentiation of Amœbæ Coli in the Tissue.—1. Harden in alcohol. Embed.

2. Stain the celloidin or paraffine sections with saturated aqueous solution of thionin, five to twenty minutes.

3. Wash in water.

4. Differentiate in a 2-per-cent. aqueous solution of oxalic acid, one half to one minute.

5. Wash in water.

6. Dehydrate, clear and mount.

The nuclei of the amœbæ and the granules of mast-cells are red. The nuclei of all other cells are blue. Cover-glass preparations are not applicable to this method. The excreta from the intestinal canal may, however, be hardened in alcohol. Similar results may be obtained with Unna's plasma cell stain.

17. Plimmer's Method for the Demonstration of Parasites in Cancer.—1. Small slices of tissue are hardened in Hermann's fluid 12 to 24 hours.

2. Thorough washing in running water, 12 to 24 hours.

3. Harden in alcohol. Embed in paraffine.

4. Remove paraffine in xylol.

5. Absolute alcohol.

6. Place in peroxide of hydrogen until the black is removed from the section and no further bubbles form upon the surface. One quarter to half an hour.

7. Wash in water.

8. Stain with Heidenhain's iron hæmatoxylin or Mallory's simple iron hæmatoxylin.

9. Thorough washing in running water 3 to 6 hours.

10. Stain in 1-per-cent. Erlich's neutral red or Bordeaux red. (This solution must be kept neutral. When it becomes acid it must be neutralized with alkali.) After staining with iron hæmatoxylin the differentiation must be continued until the protoplasm is colorless. The amount of red in the preparation must be controlled under the microscope.

Parasitic bodies in cancer and yeast organisms in the tissue are stained yellowish red to coppery red; nuclei blue black; connective tissue structures brilliant red.

Instrumentarium.

The student should provide himself with the following instruments or have them at his disposal: A freezing microtome; an ordinary razor; two or three scalpels; a fine pair of flat and a pair of curved shears; a cornet forceps; two preparation needles in handles; a small piece of emery paper for cleaning the needles; a pointed and a blunt glass rod; two section lifters, one small and one large; a small and a large camel's hair brush; several watch-glasses; several flat round glass dishes with covers; one or two porcelain paint dishes; one or two small porcelain developing trays (4 x 5); a support made of wire and a small funnel; filter paper; slides and cover-slips; several small pairs of forceps; one or two conical glasses; wide-mouthed bottles for hardening material; bottles for the necessary coloring solutions and a few drop bottles for reagents.

PART II.

PATHOLOGICAL HISTOLOGY OF ORGANS.

I. HEART MUSCLE.

Structure.—The heart wall is made up of three layers, the epicardium (or pericardium), the myocardium, and the endocardium. The epicardium covers the outer surface and consists of a connective tissue structure including a considerable amount of fat tissue and elastic fibers. The elastic fibers of the epicardium are formed into a definite layer which lies directly beneath the flattened polygonal epithelium which covers the surface and lines the cavity of the epicardial sac.

The myocardium is composed of striated muscle fibers which differ from the striated fibers of the skeletal system. The fibrillæ of the fiber are placed at the periphery and the principal mass of sarcoplasm occupies the center of the cell, embedded in which is a single long cylindrical nucleus, the ends of which terminate abruptly. The muscle fibers are much shorter than those of the skeletal muscles and are united at the ends, beside occasionally anastomosing with each other. At the point of union a narrow line of cement substance may be made out. The cardiac muscle fibers possess no sarcolemma. A certain number of fine connective tissue fibrils may be discerned between the muscle fibers. In young individuals the heart wall contains but a small amount of elastic tissue, which is usually localized about the capillaries and in the connective tissue structures in which these lie. As the age of the individual advances the amount of elastic tissue between the muscle fibers increases.

The endocardium consists of a layer of connective tissue in which a certain number of smooth muscle fibers and a considerable amount of elastic tissue are included. Upon the surface of this structure is arranged a single layer of irregular flattened polygonal endothelium. The valves of the heart are composed of dense connective tissue which, on the side of the valve exposed to the blood pressure (in the arterial valves on the ventricular side and in the venous valves on the auricular side), is richly interspersed with elastic fibers. The leaves of the normal heart valve contain no blood vessels. These extend into the valve only so far as it is composed of muscle. In the newborn the connective tissue portion of the valves is poorly developed, and the vessels appear to penetrate much farther. However, the free surface of the valves, even in this case, contains no vessels. Not uncommonly deep clefts in the valves are interpreted as blood vessels. These may, however, by employing serial sections, be seen to communicate with the surface of the valve. The so-called *valve hæmatomata of the newborn* are not produced by hemorrhage from the intrinsic vessels of the valve but are due to imperfect development leading to the formation of clefts, and have no relation whatever to the minute hemorrhages found beneath the peri- and endocardium, in cases of death from suffocation.

The pathological processes involving the layers of the heart differ so widely that we will only discuss those affecting the myocardium, leaving the epi- and endocardium to be considered under Serous Membranes and Blood Vessels.

1. Fatty Degeneration

Is the result of constitutional disorders, such as advanced anæmias, chronic infections (phosphorus poisoning), etc. The change is most easily detected in the papillary muscles, where it causes a yellowish mottling of the tissue (Tigerung). When the degeneration is marked, the muscle loses its elasticity and becomes brittle. *The fresh method affords the best means of studying the condition.* Small particles of tissue are cut from the affected portion of the muscle with a curved shear and teased in salt solution until a few fine fibers remain. (See technique of fresh preparations.) The larger pieces are discarded and the finer ones covered with a cover-glass. Examined under low power the muscle fiber appears grayish brown with occasional transverse sections which appear nearly black. The dark portions of the muscle fiber owe their loss of transparency to deposits of fat marking areas of degeneration which are scattered irregularly through the tissue. The dark areas are identical with the yellowish spots observed in the papillary muscle.

By higher magnification the periphery of the degenerated area must be examined. In the unchanged muscle adjacent to the affected portion the characteristics of the muscle fibers may be distinctly seen with their transverse and longitudinal striation. When the dark areas of the tissue are examined, the muscle fibers are seen to contain great numbers of minute highly refractive spheres, which are arranged in conformity with the longitudinal and transverse striation. (Pl. I., Fig. 1.) These highly refractive bodies are fat droplets.

The proof is not far to seek when potassium hydrate solution is allowed to flow under the cover-glass. This done and the tissue observed under a low power lens, the unaffected portions of the muscle are seen to become transparent, while the degenerated portions in comparison become darker and more sharply defined.

As we have already learned, all albuminous constituents of the tissue, when exposed to potassium hydrate solution, become transparent, only fat, bacteria and elastic fibers remaining unchanged. The examination with high power shows the fat droplets more plainly than before the reagent was applied, and the transparent condition of the tissue reveals the arrangement and highly refractive character of the drops to better advantage.

Material hardened in formaline and cut on the freezing microtome may be stained with Sudan III. when the fat droplets take on a brilliant orange red color (Technique, page 55). Permanent mounts are best obtained by hardening the tissue in a solution of osmic acid, using either Flemming's, Hermann's or Marchi's method, when the fat droplets appear black (Technique, page 55).

2. Invasion of the Heart Muscle by Fat Cells.

Here the muscle is not affected by degeneration, but is simply invaded by the pericardial fat tissue. The usual site for the invasion is the apex of the right ventricle, where the yellow fat may be seen extending into the red muscle of the ventricular wall.

For purposes of examination a piece of tissue which includes the heart muscle and the pericardial fat must be removed, hardened in formaline, and cut on the freezing microtome. The plane of the section must be perpendicular to the pericardial surface and include the fat tissue and muscle. (N. S.)

Sections hardened and mounted as above show the large fat cells invading the muscular structure.

In material which is embedded the fat is usually extracted, leaving spherical openings in the preparation representing the fat cells and indicating their previous location. The muscle fibers about the site of the invasion show marked atrophy and are commonly displaced and flattened against their neighbors. (Pl. I., Fig. 2.) The line of demarkation between the pericardial fat and the ventricular wall is irregular and indistinct.

If a fresh section of the tissue be stained with hæmatoxylin and Sudan III.

Reference:

C. Göbel. Beitrag z. fett-Deg. d. Herzens. Central. f. path. Anat., IV., 1893, Nr. 18.

and embedded in glycerine, the fat cells appear as large, brilliant, orange-red spheres between the muscle fibers.

3. Parenchymatous Degeneration (Cloudy Swelling)

Occurs in patients who have suffered from acute febrile disease. The heart muscle has a uniform grayish cloudy appearance, and is very brittle. Teased preparations from the muscle appear darker than normal when examined with low power, and to this extent resemble fatty degeneration. By higher magnification the similarity is even greater, as the muscle fibers are filled with small granules closely resembling the fat droplets. These small granules can be easily recognized as the cause of the dark appearance of the fibers under low magnification, as their great number and high refractive index obstruct the passage of light through the tissue. Occasionally the fibers are so filled with granules that the entire preparation presents a dusty appearance. Although the granules somewhat resemble fat when seen through the microscope, the addition of acetic acid or potassium hydrate discloses their true nature, as they disappear as rapidly as the reagent reaches them, proving that they are an albuminous product and not fat.

As the albuminous constituents of the protoplasm are deposited in the form of granules, when decomposition is advanced, the diagnosis of true parenchymatous degeneration can be made only when the tissue is examined shortly after death.

Parenchymatous degeneration is not uncommonly a preliminary to diffuse fatty degeneration, in which case the muscle fibers are filled both with minute fat droplets and albuminous granules, which disappear under the action of the reagent and leave the fat droplets sharply defined. In such cases the muscle has the yellowish appearance of fatty degeneration.

4. Brown Atrophy.

This is the most common pathological change found in the heart muscle, and as a senile condition is an almost constant occurrence in the hearts of the aged. As a pathological process it is the result of nearly all chronic constitutional disorders. It derives its name from the brown appearance of the muscle.

The fresh examination again suffices. (See Technique, page 19.) As the ventricular wall is uniformly affected, the specimen may be taken from any portion, provided it is of brown appearance. The examination with low power at once discloses the presence of fine, yellowish brown dots, one or two in each muscle cell, near its nucleus.

By higher magnification these dots of yellowish brown are resolved into fine, yellow granules massed together at the poles of the nuclei, usually in pyramidal form with the apices pointing in the long axes of the cells. (Pl. I., Fig. 4.) The brownish discoloration of the muscular structure is in proportion to the amount of pigment deposited.

In fresh preparations the nucleus cannot be seen until a reagent (acetic acid) is applied, when it makes its appearance as an oval body occupying the center of the muscle cell and surrounded by the granules of pigment. As compared with fat, these granules can be easily differentiated from the round, glistening, colorless fat droplets, by their angular form, yellow color, and characteristic location near the nucleus. As the pigment granules are of varying size, and sometimes appear as round, almost colorless bodies, the differentiation from fat droplets may be a matter of difficulty.

Under such conditions the principal difference is the resistance which pigment shows to various substances in which fat is soluble, *i. e.*, alcohol, xylol, ether, and oils.

Fat may be excluded in sections hardened and treated by any method in which fat extractives have been employed, whereas pigment is unchanged and appears even more distinctly as yellowish granules surrounding the nucleus.

In brown atrophy the muscle fibers are smaller than in the normal state, but show plainly the longitudinal and the transverse striation. Excellent sections may be obtained by hardening in M. F. and cutting on the freezing microtome. Stain with carmine.

In general, a brown or brownish discoloration of the tissue, whether it occurs in the liver, spleen, lung or other organ, indicates pigmentation, for which reason it is desirable to sharply differentiate between red and brown in the macroscopic examination of tissue.

5. Abscess in the Heart Muscle.

Abscesses result from infection which usually reaches the interior of the heart wall through the coronary arteries. This is most commonly the result when the aortic valves are affected by an ulcerative endocarditis, their location directly before the openings of the coronary arteries being favorable to the entry of detached septic fragments into the coronary circulation.

The organisms which produce malignant endocarditis and the associated abscesses of the heart muscle are, in by far the greater number of cases, the ordinary pus-forming staphylococci and streptococci (occasionally gonococci).

Macroscopically the small abscesses appear as minute yellow spots each surrounded by a red zone and distributed along the main axes of the muscle fibers. These two characteristics (red zone and arrangement between the fibers) serve to distinguish them from simple areas of fatty degeneration.

The abscesses are most commonly located beneath the endocardium of the ventricular wall and in the papillary muscles. In the center of the abscess a minute white point or line may be occasionally discerned.

M. F., F. M., or where larger abscesses are present the material must be embedded in paraffine or celloidin. Carmine and gram or methylene-blue.

In stained sections low magnification shows deeply stained areas in the muscle which correspond in size and arrangement to the yellow spots seen with the naked eye in the fresh tissue. (Pl. I., Fig. 5.) These are of varying size, nearly all of them have a larger or smaller unstained center surrounded by a deep blue zone. If the center of the focus is examined with high power, it is found to consist of unstained material which resembes the muscle fibers in form and arrangement. In this region no nuclei may be made out, but between the unstained muscle fibers are found deeply stained masses. By examining the edges these masses are resolved into deeply stained, closely packed, minute spherical bodies of uniform size. The spherical form, uniform size and specific color reaction, indicate that they are micrococci.[1]

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[1] It is well to observe that in sections stained with Gram's method or methylene-blue, similar masses of deeply stained granules may be found in the connective tissue, which on first appearance resemble very closely masses of micrococci. Careful examination, however, shows certain differences. First, the small spherical granules are not so closely packed as in the masses of organisms. Secondly, these masses of deeply stained granules are found to be scattered with comparative regularity through the connective tissue structure. Thirdly, the masses are more or less of the same size. Fourthly, there are no evidences of necrosis in the adjacent tissue. Fifthly, careful examination of the mass determines the presence of a nucleus at the center of each of these accumulations of granules. In fact, we are here dealing with a special form of granulated connective tissue cell, the so-called mast cell. The minute spherical bodies are the granulations of the protoplasm of the cell, which are arranged about the nucleus. These granules stain regularly with methylene-blue and show a variable reaction in preparations stained with either Gram's or Weigert's fibrin method.

The unstained material at the center of the abscess, which appears in the form of more or less distinct structures representing the muscle fibers or granules and flakes of disintegrated tissue, is the result of the action of toxic substances elaborated by the organisms. This destruction of the tissue is known as necrosis. Surrounding these necrotic centers is found a deeply stained zone which is equivalent to the yellow portion of the abscess when viewed macroscopically.

The examination of this zone with high power shows the deep coloration of the tissue to be due to countless round cells which possess curved, horseshoe-shaped or clover-leaf nuclei. In form and size they are exactly like the majority of the white blood corpuscles, the so-called polymorphonuclear leucocytes. In fact, the so-called pus cells are for the greater part leucocytes, which, under the action of acute irritants, pass out through the walls of the vessel and accumulate about the source of irritation. These are the so-called exudate cells (hæmatogenous migrating cells). The masses of organisms and the tissue which they are destroying are surrounded by a closely packed zone of these cells, which, in a certain sense, form a protecting wall against the ravages of the organisms and their toxic products. Occasionally these pus cells take the organisms into their bodies and disintegrate them. This is the so-called phagocytic action of the leucocyte.

Where the pus cells are most closely packed the muscle fibers are found to be entirely destroyed, which is probably due to a form of digestive action brought about by the excretory products of the leucocytes. In the necrotic center of the abscess occasional leucocytes will be met. Where these come in direct contact with the organisms, or are exposed to the action of their toxic substances, they likewise fall victims to the general necrosis. The remains of the nuclei of the leucocytes thus disintegrated are found in deeply stained granules and spheres of chromatin disseminated through the necrotic tissue.

The tissue adjacent to the abscess is thickly infiltrated with pus cells, which lie between the muscle fibers. It is by this infiltration that the abscess grows in size. Surrounding the zone of purulent infiltration is a second hemorrhagic zone which gives the abscess its red border when seen macroscopically. Examination with low power shows the capillaries of this region greatly congested and filled with red blood globules (inflammatory hyperæmia), while scattered between the muscle fibers are great numbers of free blood corpuscles (inflammatory hemorrhage). (Pl. I., Fig. 5.)

The zone of hyperæmia and hemorrhage is the result of the toxic action of the organisms which causes not only irritation of the vessels but direct injury to their walls, permitting the escape of the cellular elements of the blood into the surrounding tissue.

By studying the structure of an abscess, we may form some idea of the mechanism of its formation. The presence of the pus-forming organisms in the tissue produce, first, irritation, and then necrosis of the adjacent structures. Around this center of irritation accumulate the leucocytes which pass out through the walls of the adjacent hyperæmic vessels. The method by which the organisms find their way into the tissue may not always be determined by the examination of a single abscess. A careful search of the section will disclose one or more capillaries plugged with organisms. The walls of the capillaries and the adjacent muscle fibers do not always show necrotic change or the accumulation of leucocytes which constitute the well-formed abscess. We have therefore detected the beginning of the local process. When the abscess is formed and the tissue has become necrotic and infiltrated with leucocytes, this relation of the organisms to the vessels is obliterated, and the multiplying organisms soon spread through the surrounding tissue. It occasionally occurs, however, that branching masses of cocci which show distinctly that they have lodged at a point of bifurcation in a capillary, may be seen in small abscesses. Every small abscess does not contain a necrotic center. In these cases the absence of necrosis is probably due to the modified virulence of the organism.

The group of organisms is invariably surrounded by an accumulation of leucocytes which infiltrate the tissue and to some extent obscure the necrosis.

The fresh examination of double knife sections is extremely instructive. The best sections are obtained from the papillary muscles when these contain abscesses. On the addition of acetic acid to the fresh section, the abscesses appear with a clear center, in which are minute brownish dots, and without the clear center, a wide dark zone. The center is composed of necrotic muscle fibers which, under high power, are resolved into masses of detritus. The brown dots are now seen to consist of masses of bacteria, lying in the necrotic mass. The dark zone is found to be due to an excessive fatty degeneration of the surrounding muscle fibers. This zone is equivalent to the deep blue zone of purulent infiltration in hardened and stained sections. The fatty degeneration is the result of disturbance of the metabolism of the living muscle fibers, following partial obstruction of the local blood supply. The zone of hyperæmia and hemorrhage so readily observed with the naked eye in the fresh tissue and under the microscope in the stained sections, is not demonstrable in fresh sections treated with acetic acid, which causes rapid disintegration of the blood globules. The cause of the abscess, the bacteria, are only visible when specially stained (methylene-blue, Gram's method), when they are usually found near the center of the abscess, in densely packed masses.

6. Myocarditis Chronica Fibrosa

Is generally the result of obliteration of one of the finer branches of the coronary arteries through sclerotic thickening. It is most commonly found in the anterior wall of the left ventricle, near the apex, and in the posterior wall near the base. It seldom occurs in the right ventricle.

Microscopically the areas affected appear as whitish spots in the heart muscle, often extending through the entire thickness of the ventricular wall. The smaller foci commonly occur directly beneath the epicardium as pale spots shining through the membrane. The irregular outlines of the degenerated areas are sharply defined against the red heart muscle. M. F., F. M., N. S. or van Gieson, elastica stain, or Mallory's connective tissue stain.

Low magnification reveals a palely stained area. Pl. I., Fig. 3, shows such a focus by medium magnification. The unstained portion consists of fine fibrous connective tissue with an occasional isolated and atrophic muscle fiber.

By high power the clear portion of the tissue is found to consist of a fine, fibrillated, wavy, connective tissue, possessing delicate and elongated nuclei, with here and there an occasional capillary. As we approach the periphery the cellular elements grow in number until at the margin these have so increased that we may speak of a round cell infiltration. These round cells belong to the class of migrating cells, are smaller than pus cells and very similar to the round cells found in lymph nodes and the lymph circulation, and closely resemble the small, mononuclear leucocytes of the blood. Their nuclei are large, occupying nearly the entire cell body, and stain intensely.

The derivation of these cells is still a matter of doubt; in all probability they spring from more than one source. Many come from the connective tissue nuclei, while others are derived from leucocytes. They correspond very closely in appearance and size, so that it is impossible to say to which they owe their origin. Whether or not they are altered leucocytes which have migrated from the vessels is difficult to determine. Some observers believe that they are derived from the lining endothelium of the lymph channels and capillaries while others have attempted to trace their derivation from a special form of connective tissue cell which plays a part in the formation of the adventitia of the vessels. In the fœtus and newborn these cells show an unusual chain of transition forms, including one which apparently possesses free movement.

According to this theory the cells of the adventitia surrounding the vessels change into large round cells and these by proliferation produce the small lymphocytes which form the greater part of the accumulations of cells known as round cell infiltrations. There is nothing particularly improbable in the hypothesis that this form of migrating round cell is formed from the cells of the adventitia and the large round cells of the newborn, in case these are proven to be identical with the primary migrating cells, which are scattered everywhere through the connective tissue of the embryo. According to Saxer, red blood globules and the various forms of leucocytes are derived from the migrating cells of the embryo.

Although it is improbable that round cells possess the ability to form connective tissue, they are at least regularly associated with chronic inflammatory processes of that structure. Their presence at the periphery of the fibrous area in the heart muscle reveals the inflammatory nature of the process.

The muscle fibers at the periphery and such as extend into the affected area are atrophic and contain pigment. As we approach the center we find the muscle fibers entirely replaced by connective tissue, with only an occasional group of pigment granules to suggest their previous existence.

The process is one of complete destruction of the muscle in a given area, the result of disturbance of metabolism following occlusion of an artery or direct injury to the tissue, and repair of the defect by connective tissue, with the formation of a scar. The older the fibrous area, the richer it becomes in elastic fibers until, in advanced cases the entire scar appears to be composed of elastica (special stain). Whether there is any attempt on the part of the unaffected muscle fibers to replace the destroyed tissue is a point not readily determined. At any rate, it is but slight the only appearance at all suggestive of such an effort being the enlarged nuclei often found in the neighboring healthy fibers. Beside the nuclei, in such cases the muscle fibers are often enlarged, and it is quite probable that the enlargement of nuclei and fibers is merely a form of compensatory hypertrophy, due to excessive work thrown upon the surrounding muscular elements.

In very rare, comparatively fresh cases of necrosis of the heart muscle, budding of the adjacent muscle fibers has been observed. These changes have been compared to the process of regeneration which occurs in the skeletal muscles. By all means the most common cause of chronic myocarditis is closure or excessive diminution of the lumena of the peripheral branches of the coronary arteries. The resulting diminution of the blood supply brings about the destruction of the groups of muscle fibers which are nourished from the affected vessel, and the necrotic process proceeds in exactly the same manner as that which brings about the formation of anæmic infarcts in the kidneys. When necrosis is complete the tissue is organized in exactly the same manner as are thrombi, that is, through the growth of young connective tissue, but as the

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stages of necrosis and beginning organization are but seldom observed, they will not be considered at this time. A portion of the muscular wall becomes necrotic when the afferent vessel which supplies it is plugged by a simple embolus, in which case the processes of necrosis and organization follow in exactly the same manner as when the vessel is closed by any other occluding agent. Abscesses which are followed by resolution, leave scars in the heart muscle which are not ordinarily distinguishable from those resulting from other causes. Necrosis of the muscle fiber may likewise result from the action of toxic substances (bacterial poisons) in which case the surrounding muscle shows evidence of degenerative changes, the principal of which are (for instance, in diphtheria) fatty, vacuolous, and hyaline.

Lastly, the degeneration of the tissue may result from primary changes in the connective tissue. In this last form should be properly included the more or less superficial fibroid areas found directly beneath the peri- or endocardium. These are usually associated with areas of chronic pericarditis or endocarditis and the degeneration of the muscle is very probably produced by proliferation of the connective tissue between the muscle fibers, leading to secondary atrophy and destruction.

In spite of the great variety of causes which may lead to the formation of scar tissue in the heart muscle, the mature scars are so similar that very little may be determined as to their origin. Beside the formation of isolated areas of fibrous tissue in the heart muscle, diffuse proliferation of the connective and elastic tissue are occasionally encountered. The etiology of the diffuse forms is obscure.

7. Tuberculosis of the Heart Muscle.

This, as well as syphilis, is a rare affection. The recognition of miliary tubercles in the heart wall is of importance, as macroscopically they closely resemble small, multiple abscesses, in that they appear as minute gray spots distributed in the long axes of the muscle fibers, and at times are surrounded by red zones, in which case the establishment of a differential diagnosis is difficult.

F., F. M., N. S. Tubercle bacilli stain. Weigert's fibrin stain.

By lower magnification the tubercle appears as a dark spot with a colorless center. Here and there can be distinguished deeply stained masses of protoplasm usually distributed between the clear center and the dark peripheral zone.

Higher magnification shows the minute structure of the tubercle. The dark peripheral zone consists of cells of varying form and appearance, first, large oval and spindle cells, with oval, poorly stained nuclei, lying usually nearest the unstained center, which, because of their similarity to epithelial cells, are known as epitheloidal cells, and which are derived either from the connective tissue or endothelium.

Secondly, the usual small round cells, scattered between the epitheloidal cells and toward the periphery, where they often form a separate zone. (Compare Plate XXII., Fig. 5.)

The tubercle may be looked upon as a chronic inflammatory focus in the connective tissue. It is usually small (size of millet seed), when it is known as a miliary tubercle, still smaller tubercles bearing the name of submiliary. The center of the tubercle never contains blood vessels or capillaries.

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The cellular elements so far described are not necessarily characteristic of tuberculous processes, the distinguishing feature of the tubercle being the unstained center. Examination at this point shows first an entire absence of cells of any sort, the whole consisting of a structureless mass, at times appearing somewhat granular. It represents the yellow spot seen in the fresh state, which, because of its resemblance to cheese, is designated as "caseous" material. This is the remains of the cells destroyed by the excitant of the process, the tubercle bacillus.

Where the bacillus finds lodgment it causes, through production of an irritant poison (ptomaine), a chronic proliferation of the connective tissue. The cellular elements thus formed succumb to the action of the bacillus, and, disintegrating, form the caseous material at the center of the tubercle. The tubercle spreads peripherally, continually destroying the wall of cells which the tissue throws up as protection against the invasion. As the tissue perishes it becomes infiltrated with lymph and is transformed into the hyaline, caseous mass which forms the central portion of the tubercle.

Tissue which contains very fresh, young tubercles, when stained with Weigert's fibrin stain, reveals the presence of true fibrin, within the areas of caseation, showing that the tubercle bacillus is capable of producing exudation into the tissue of the fibrin-forming elements of the blood.

At the junction of the caseous material and the peripheral cellular zone, are again found the deeply stained masses of protoplasm seen in this location with low magnification. They are the so-called giant cells, containing large numbers of deeply stained nuclei, often arranged near the periphery. A transverse section of such a cell would naturally show some of the nuclei near the center. Often the nuclei are arranged along one side of the cell only. (See Plate XXIII., Fig. 5.)

The protoplasm of these cells is very indistinct at the margins, appearing to merge into the caseous material. Where the section is properly stained, tubercle bacilli are found in and about the giant cells and should be sought for in this locality. One might well imagine that the giant cell represented an attempt on the part of the tissue to destroy the bacilli; but the attempt is always an unsuccessful one, as the giant cells, as well as the surrounding tissue, succumb to the general caseation.

Tubercles occasionally heal when the defect is repaired by connective tissue. The structure of the tubercle varies according to the organ or tissue in which it develops as well as the virulence of the organism which produces it.

The different forms of tubercles will be considered under Tuberculosis of the Liver, Kidney, Lungs and Central Nervous System.

8. Fragmentation of the Myocardium.

Fragmentatio cordis. Myocardite segmentaire. (*Renaut.*)

This condition is found after sudden death, or profound infectious disease. The diagnosis can be made macroscopically when the autopsy follows shortly after the exodus, by the softness and fragility of the left ventricular myocardium. The breaking up of the muscle is very probably due to abnormal contraction during the death agony. *A section made with a section razor from the fresh muscle,* transferred with care to a slide, examined in salt solution under a cover-glass to which no pressure has been applied, will show with low magnification the characteristic appearances which accompany the change.

At the edges of the section the muscle fibers are isolated and detached, a result to be obtained in normal muscle only after the most careful teasing. In the center the fibers are in the greatest confusion, with their long axes pointing in all directions. In cases less marked, only a widening of the line where the ends of the muscle cells are cemented together, may be observed, with here and there partial or complete divulsion of the fibers.

Hardened and stained sections show that the fibers are not always separated at the line of juncture, but that the cell is often torn through its substance and that the nuclei are never divided. The fact that the nuclei are never torn shows that the separation of the fibers is not caused by cutting the tissue. (Plate I., Fig. 4.) Pigmentation, fatty degeneration and albuminous degeneration accompany the condition.

DESCRIPTION OF PLATE I.

FIG. 1. Fatty degeneration of heart muscle. (Moderate Degree.) See p. 67. Osmic acid preparation. Oil immersion.

The longitudinal and cross striation of the muscle fibers is plainly visible. Embedded in the substance of the fibers and scattered between them are a large number of fat droplets of varying size. The fat droplets are blackened by the action of the osmic acid.

FIG. 2. Invasion of the heart muscle by fat tissue. See p. 68. Middle power. Hæmatoxylin.

Above may be seen the sharp dividing line between the panniculus and the myocardium. The muscle fibers are separated by round and irregularly shaped spaces which previously contained fat cells. The outlines of the fat cells may be definitely made out. The contents of the cells have been removed during the embedding process.

FIG. 3. Fibrous myocarditis. See p. 72. Low power. Alum carmine. The light groundwork consists of fine fibrous connective tissue with minute deeply stained nuclei. Here and there a fine capillary. About the periphery and extending into the fibrous area are a number of deeply stained atrophic muscle fibers.

FIG. 4. Fragmentatio cordis. See p. 75. High power. Alum carmine. The muscle fibers are widely separated from one another, in many cases torn through their substance. Fine, dark granules (in section brown) are seen within the muscle cells grouped at the poles of the nuclei. A somewhat coarser deposit of pigment is seen near the center lying free between the muscle fibers. Transverse striation visible under *magnifying glass.*

FIG. 5. Abscess in muscle. See p. 70. Middle power. Hæmatoxylin. In the center of the figure a closely packed mass of leucocytes. At the periphery these penetrate between the muscle fibers. At the center of the abscess the muscle fibers are destroyed. About the periphery of the figure (magnifier) particularly at the right and above a number of red blood corpuscles between the muscle (fibers hyperæmic hemorrhagic zone).

FIG. 6. Pigment atrophy of the liver. See p. 82. High power. Alum carmine. Center of a liver lobule. The central vein surrounded by the characteristic network of liver cells. Under the *magnifier* the cells about the central vein are seen to contain, beside the deeply stained nuclei, a number of fine, angular granules (in the section yellowish brown). The capillaries between the liver cells are considerably dilated, but contain no blood corpuscles, as these have been removed during the process of hardening and embedding.

II. SKELETAL MUSCLES.

Structure.—The fibers of the skeletal muscles are composed of long, cylindrical cells, which possess a distinct, structureless membrane, the sarcolemma. The contractile portion of the cell is composed of fibrillæ which show longitudinal and transverse striation. Between these lies the sarcoplasm. The nuclei are placed directly beneath the sarcolemma, seldom in the center of the cells.

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PLATE I.

Fig. 1.

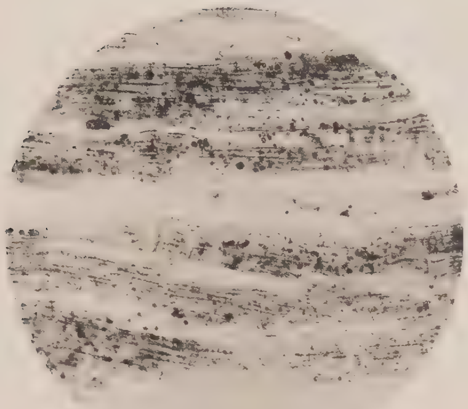


Fig. 2.

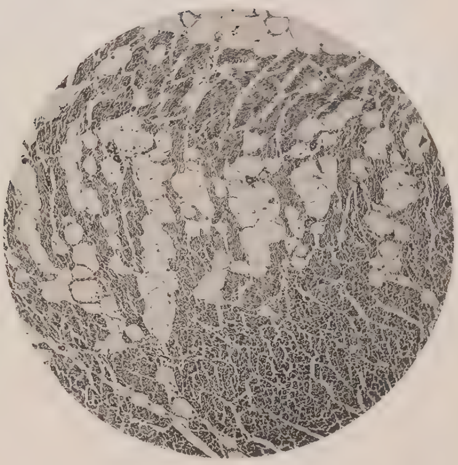


Fig. 3.

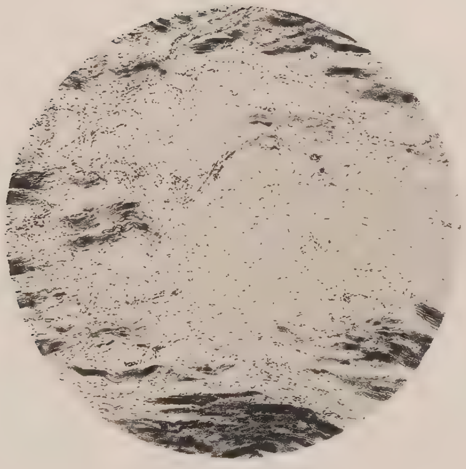


Fig. 4.

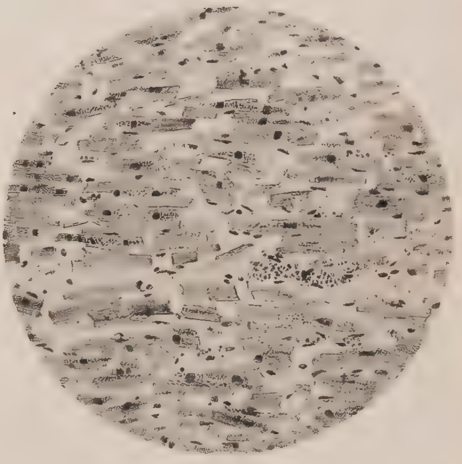


Fig. 5.

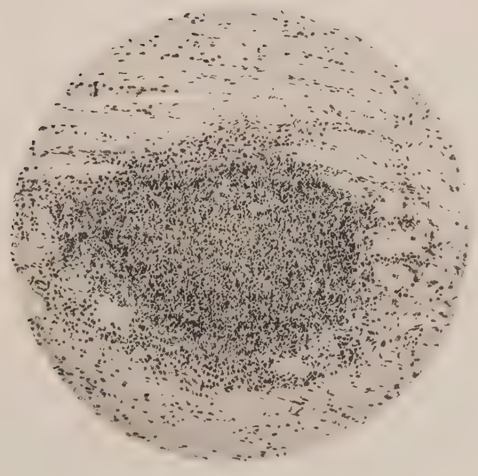
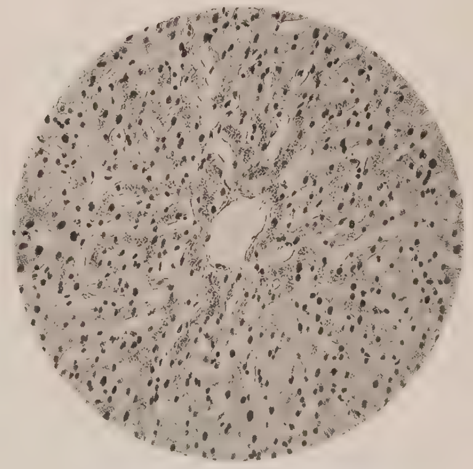


Fig. 6.



Gaylord & Aschoff — Pathological Histology.

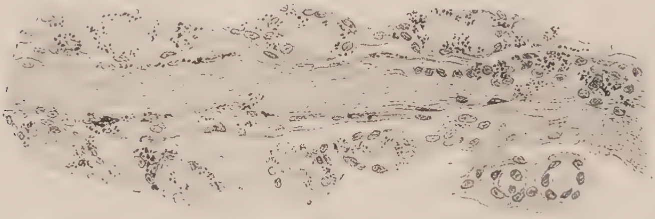
Heliotype Co. Boston.

Among the forms of degeneration to which the skeletal muscles are subject are parenchymatous and fatty degeneration. The changes which these degenerations induce in the skeletal muscles differ in no way from the same processes in the heart muscle.

1. Atrophy of the Muscle, from Loss of Function.

In diseases of the nervous system, or where the muscle is inactive as in amputation stumps, the muscle fibers undergo atrophic change, which gives it a pale or brownish appearance. Microscopic examination of either fresh teased preparations, or hardened and stained sections, shows that the fibers are smaller than normal and affected by fatty degeneration and brown atrophy. The nuclei of many of the cells have apparently proliferated, as the sarcoplasm contains several nuclei which are usually distributed in the peripheral portions of the cell.

FIG. 9.



Atrophy of muscle fibers from inactivity. Some of the fibers have broken down into masses of protoplasm containing proliferated nuclei. Extensive deposits of pigment.

Beside this increase in the number of nuclei in the muscle cells, we find large masses of protoplasm within the sarcolemma which contain nuclei and present the appearance of giant cells. (Fig. 9.)

This proliferation of nuclei and isolation of masses of protoplasm in the form of polynuclear giant cells is also found in regenerating muscles after surgical or other wounds. The process may, therefore, be viewed as an attempt on the part of the muscle at regeneration, which, owing to the unfavorable conditions (inactivity, impaired nutrition) has been ineffective. The longitudinal and transverse striation of the atrophic fibers, is usually preserved.

2. Progressive Muscular Atrophy.

(*Atrophia musculorum lipomatosa pseudo-hypertrophica.*)

Primary, progressive muscular atrophy or dystrophy is not the result of disease of the nervous system or inactivity, but is more probably induced by a pathological increase of the connective tissue of the muscle, or is a primary affection of the muscle fibers.

Formaline, F. M., N. S. or Sudan III., or harden in Flemming or Hermann. Embed in paraffine or celloidin. Both transverse and longitudinal sections should be made. Stain with safranin or iron hæmatoxylin; or embed in Zenker, stain with Mallory's connective tissue stain.

The most prominent histological feature of the process is the intermingling of small (atrophic) and gigantic (hypertrophic) muscle fibers, best seen in cross sections of the muscle. The atrophic fibers show various forms of degeneration.

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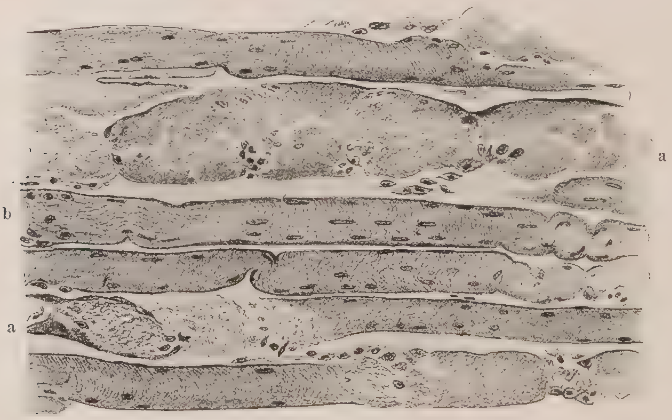
Beside the variation in size of the fibers, we find a great increase in the connective and fat tissue, which in many places appears to invade the muscle fibers. In the ordinary atrophy from loss of function there is no special increase in the fat and connective tissue which supports the muscle fibers. In progressive muscular atrophy the increase in the fat and connective tissue is so great that, in spite of the advanced atrophy of the specific fibers, the muscles are increased in size and macroscopically appear hypertrophic. Examine fresh teased preparations or harden in formaline, F. M. Transverse or longitudinal sections. N. S., van Gieson.

3. Waxy Degeneration of the Muscle

Occurs after severe burns or injury of the tissue from strong chemicals, and as the result of acute febrile conditions, as typhoid fever, when the change is most commonly found in the recti muscles of the abdominal wall and in the adductor muscles.

Examination of fresh teased preparations by low magnification shows occasional fibers of normal appearance, in which the striation, both longitudinal and transverse, is preserved. Intermingled with these are fibers containing large, glistening, irregular plates with cleft edges. (Fig. 10.) These masses are the result of a form of coagulation of the sarcoplasm, and their appearance justifies the term waxy. The degenerated masses do not entirely fill the sarcolemma sacs, and a clear space can often be demonstrated between the periphery of the cell and the cell contents. If the process has existed for a prolonged period of time, multinuclear masses of protoplasm appear within the sarcolemma. These masses of protoplasm contain fragments of coagulated tissue, and are derived from portions of the sarcoplasm which have escaped the effects of the degeneration. Their appearance indicates the beginning of the regeneration of the destroyed muscle fibers.

Fig. 10.



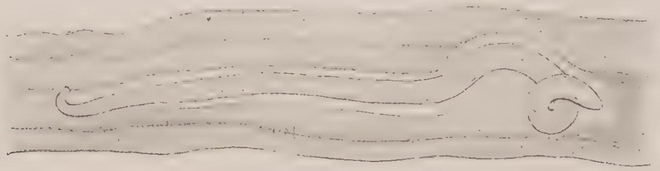
Waxy degeneration of muscle fibers, a, degenerate fibers ; b, preserved fibers.

The purulent, inflammatory processes in the skeletal muscles differ in no way from those described under "heart muscle."

4. Trichina Spiralis.

This, the most common and dangerous parasite which invades the muscular structure, belongs to the class of worms known as Nematodes. The muscle trichina varies from $\frac{7}{10}$ to 1 millimeter in length, has a blunt head and pointed tail, and is the young or larva of the trichina intestinalis. (Fig. 11.) The intestinal form of the parasite is larger than the muscular form, the female measuring 3 millimeters; the males are about half that length. The young worm is expelled from the uterus of the female into the lymph channels of the intestinal wall, and thence to the remoter parts of the body. The exact method of its migration is not known, but its ultimate destination is the muscular structure, the localities most commonly affected being the diaphragm, pharynx, muscles of the neck and the skeletal muscles.

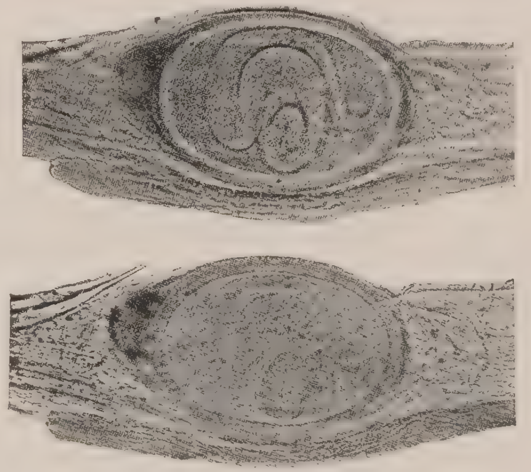
Fig. 11.



Migrating trichina in muscle.

Having reached the muscle, the trichina bores through the sarcolemma and enters the muscle fiber, where it destroys the sarcoplasm and ultimately coils itself into a spiral, the surrounding sarcolemma forming a capsule which, owing to the prominent projection of the poles, appears like a miniature lemon. (Fig. 12.) Later a deposit of lime salts makes its appearance at the poles, and gradually spreads until the entire capsule becomes calcified.

Fig. 12.



Photomicrograph of an encapsulated trichina; a, in the plane of the capsule showing calcification; b, in the plane of the worm. Calcified polar mass to the left.

Surrounding the sarcolemma capsule the connective tissue forms a second capsule, at the poles of which a deposit of fat globules is usually found. When the infected muscle is eaten by an animal the capsule is dissolved by the acid gastric juice, and the freed trichinæ develop in the intestinal tract until maturity is reached, when the female bores into the villi and deposits the embryos in the chyle vessels (Askanazy), by which they are transported into the body.

As to the length of time taken in the different phases of development, the following is known: The trichina reaches maturity on the second or third day after its liberation from its capsule in the infected tissue. By the seventh day the females begin giving birth to the embryos and bore into the mucosa of the intestine. The embryos enter the chyle vessels and are transported into the body. By the fourteenth day they have entered the muscle. Calcification of the capsule begins at about the sixth month. (Fig. 12.) In the encapsulated stage the trichina may lie quiescent for years. When the parasites perish their bodies are calcified with the capsule.

The examination of muscle for trichinæ is best accomplished in the *fresh state.* If the trichinæ are calcified they may be detected as minute white bodies distributed along the red muscle fibers, and experience has shown that they are most often present at the junction of the muscle and its aponeurosis, and often only at this point. One of the muscles most commonly affected, *e. g.*, the diaphragm, having been selected, an incision made at the aponeurotic junction, the suspected trichina capsule is removed with a shear, and after careful teasing to remove the surrounding fibers, is examined in salt solution. By low power the lemon shape of the capsule is apparent, and the dark masses at the poles are due to the deposit of calcium salts. (Fig. 12.) When the calcification is complete, the whole capsule appears dark. In the center of the capsule may be seen the trichina coiled in a spiral. Occasionally the capsule contains two or more worms. By higher power the dark masses of lime salts are resolved into fine granules. That these are not fat may be determined by allowing a 5-per-cent. solution of hydrochloric acid to flow under the cover-slip, when they gradually disappear. In this manner totally calcified capsules may be cleared, disclosing the trichina. Often beside the calcium granules the above-mentioned deposit of fat may be seen at the poles, which persist after the application of the hydrochloric acid.

III. THE LIVER.

Structure.—The liver is composed of a series of cylindrical systems of cells. These are arranged in apposition. In the axes of these columns of cells run the terminal hepatic veins. Between the columns are three or four triangular spaces which are filled in with connective tissue. In these run the terminal branches of the hepatic arteries, the gall ducts and the portal veins. The connective tissue of these areas is derived from the connective tissue surrounding the larger portal vessels and is known as the periportal connective tissue. When these columns of liver cells are cut transversely, they present a section which is known as a liver lobule. Lobules are difficult to demonstrate in the organs of young individuals, but in advanced age or under pathological conditions they may be recognized because of the difference in color between the periphery and center of the lobule. Under low power the arrangement of the liver lobules may be readily determined. For the greater part they come in direct contact with each other at the peripheries and are only distinctly separated at the triangular spaces occupied by the periportal connective tissue. If two or more periportal areas are connected by a circle, all the tissue within the circle practically represents a liver lobule. In the center of the lobule is seen a large vessel, which possesses no distinct wall, but which is in contact with the adjacent epithelial cells. This is the so-called central or hepatic vein. Into this vein empties the capillary system of the lobule. The capillaries have a radiating arrangement from the center outward and receive their blood supply from the hepatic arteries and portal veins. In every periportal connective tissue area are found three vessels, a branch of the portal vein, hepatic artery, and a small gall duct. The periportal connective tissue is poor in elastic fibers. The finer structure of the liver lobule is as follows: The network of liver cells has a radiating arrangement from the center toward the periphery and is accompanied and surrounded by wide capillaries. There is no coarse connective tissue structure to the lobule. The walls of the capillaries are supported by a fine lattice work of connective tissue fibrils. (See Fig. 13.) Between the walls of the capillaries and liver cells are found the so-called stellate cells of Kupffer. These send out prolongations which penetrate between the liver cells. They are generally considered of connective tissue origin, but recently Kupffer has expressed the belief that they are derived from the capillary endothelium (Arch. f. mikr. Anat., May, 1899), and do not lie without the capillaries but are a part of the capillaries. Within the network of liver cells lie the bile capillaries which are not ordinarily visible. Toward the periphery these bile capillaries lie between the liver cells and the venous capillaries, and unite to form the smaller gall ducts, which are lined with low cubical epithelium.

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1. Cyanotic Atrophy.

The general cyanosis associated with pulmonary diseases, advancing age or reduced activity of the heart, is nowhere more quickly evident than in the liver. The stasis in the venous circulation almost immediately affects the central veins and the adjacent capillaries, causing dilatation and congestion. On section of the liver this engorgement is plainly evidenced by the reddened appearance of the centers of the lobules. In advanced valvular disease of the heart this central stasis produces a dark blood red appearance about the central veins. The increase of blood in the liver is shown by the amount of fluid which flows from the tissue when it is incised, permitting the central portions of the lobules to collapse, giving the cut surface a pitted appearance. Where the cyanosis is moderate, the sharp contrast between the periphery and the center of the lobule enables us to observe the structure of the liver with great exactness. Where the cyanosis is advanced the engorgement of the capillaries extends to the periphery of the lobule, and obliterates the characteristic marking of the earlier stages of the process. The entire liver tissue in these cases is a uniform red and the lobular structure is no longer visible to the naked eye. Here and there in the red tissue minute, light colored points may be detected, which are the periportal connective tissue areas. The natural tendency is to look upon these dots as the centers of the lobules, and the red surrounding tissue as the periphery. This error must be avoided. After section the congested liver diminishes somewhat in size. *Sections should be made from the fresh tissue with the section razor*, and if the cyanotic congestion is moderately advanced the topography of the tissue may be readily determined by low power. Thus examined, the location of the centers of the lobules may be immediately detected by the red coloration.

The periphery of the lobule not uncommonly presents a dark, opaque appearance. If the tissue be thoroughly rinsed in water, or treated with dilute acetic acid, the red center of the lobule clears and the supporting structure may be made out. After the disappearance of the blood, the center of the lobule is found to consist of widened capillaries and between these narrow ridges of flattened liver cells, many of which present a yellowish-brown appearance. Where the cyanotic process is advanced, nothing is left of the normal liver structure. The cells have either entirely disappeared or only a few irregular, deformed and pigmented cells remain. The yellowish brown color of the atrophic liver cells is produced by pigmentation resulting from pressure exerted upon them from the over-filled capillaries (brown atrophy).

If the dark and opaque appearance of the periphery remains after the addition of acetic acid, it is due to deposits of fat (see Fat Infiltration). Hardened and stained sections are best suited for purposes of orientation. The material to be hardened must be placed in the fixing fluid immediately on removal from the organ to reduce to a minimum the leakage of blood from the tissue. Harden in formaline, cut on the freezing microtome. Carmine, or hæmatoxylin-eosin.

The engorgement of the capillaries at the center of the lobule, the atrophy and destruction of the parenchyma, all appear more sharply defined than in the fresh preparation. (Plate III., Fig. 1.) Not uncommonly in cases of cyanotic atrophy the periportal connective tissue is increased in amount. (Cyanotic induration. See Cirrhosis.) In most cases of cyanotic atrophy of the liver, occasional undegenerate lobules may be found scattered through the dark red tissue of the organ. These isolated lobules or groups of lobules have a lighter color than the surrounding tissue, and the microscopic examination shows them to be made up of normal or enlarged liver cells, unaffected by the general cyanosis. They probably represent a form of compensatory hypertrophy (hyperplasia).

2. Brown Atrophy.

Brown atrophy of the liver is one of the most common pathological changes to which this organ is subject. It occurs physiologically as a senile change and results in almost all cases of wasting diseases, especially starvation (carcinoma of the œsophagus or stomach), and is associated with all cases of advanced cyanotic atrophy of the organ.

Examined with the naked eye the tissue presents a distinct brown coloration and, on section, all variations of color from a distinct brown to red. Careful examination of the cut surface shows that the lobules are diminished in size and that the periportal connective tissue areas are markedly distinct. Fresh examimation of double knife sections suffices. Examined under low power the central portions of the lobules stand out with a distinct brownish coloration, the contrast between center and periphery distinctly indicating the position and arrangement of the lobules. The lobules are, as a whole, smaller than normal, and the unstained periportal areas are unusually distinct. Occasionally the centers of the lobules are likewise darker than at the peripheries (due to central deposits of fat). Where the atrophy of the tissue is the result of cachexia and is not complicated by cyanotic congestion, the distribution of the pigment is more uniform.

After clearing with acetic acid and on examination with higher magnification, the alteration in color at the center of the lobules is found to be due to deposits of brown pigment in the liver cells. The investigation of isolated cells reveals clearly the location of the pigment. This is easily accomplished by scraping and examining a small amount of fluid from the cut surface of the liver, when the cells are found swimming free and the brown pigment is plainly seen in the cell protoplasm. (Plate II., Fig. 1.)

The pigment in the cell is usually somewhat obscured by numerous fat droplets, which are readily recognized by their spherical, glistening and colorless appearance. The intercellular pigment of brown atrophy is a product of disintegration of the cell protoplasm, and is not identical with pigment deposited from the blood. Hæmatogenous pigment differs from autochthonous pigment by containing iron, which may be readily recognized with proper chemical reagents. The iron reaction fails in old deposits of hæmatogenous pigment, but in fresh deposits it is present, thus distinguishing this pigment from that of brown atrophy, in which the reaction fails at all times.

The changes of brown atrophy may be well demonstrated in hardened sections, especially those *stained with carmine*, where the brown pigment stands out plainly against the cell protoplasm. The decrease in size of the liver cells and distribution of the pigment in the central areas of the lobule are easily demonstrated. (Plate I., Fig. 6.) This characteristic location of the pigment is perhaps due to stasis in the capillaries immediately adjacent to the central veins, the result of general venous engorgement associated with cardiac disease, or advanced age, which, through pressure or by impairing the nourishment of the cells, causes the atrophy and deposition of pigment. Wherever the liver cells are exposed to pressure (in the neighborhood of tumors, by tight lacing, etc.), we find localized areas of brown atrophy.

3. Icterus of the Liver.

The third process which gives a characteristic coloration to the hepatic tissue is icterus, a condition brought about by obstruction of the gall ducts. In the larger ducts the cause may be occlusion from stone, tumor or parasites, and in the smaller ducts inflammatory change in the periportal connective tissue. In

PLATE II.

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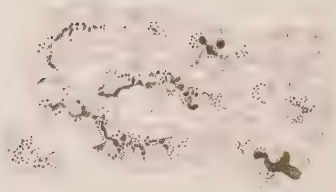

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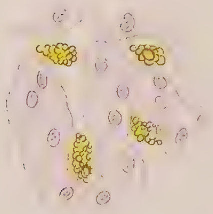

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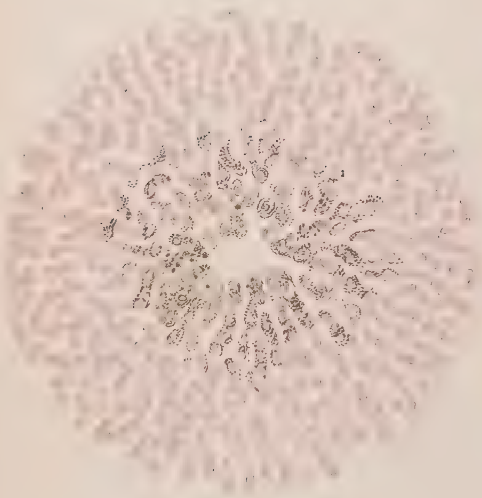

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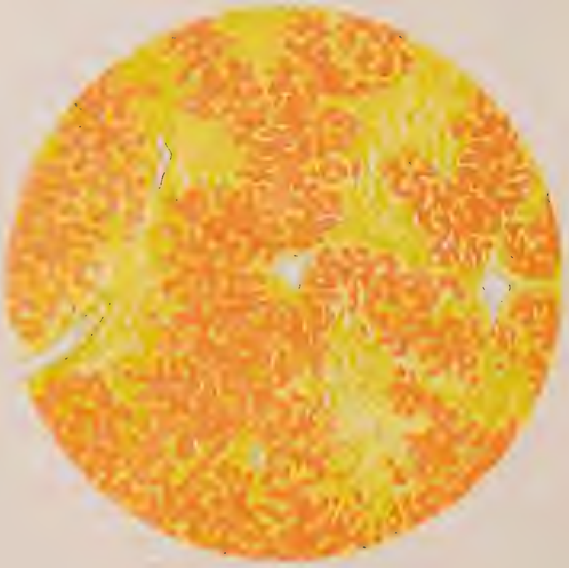

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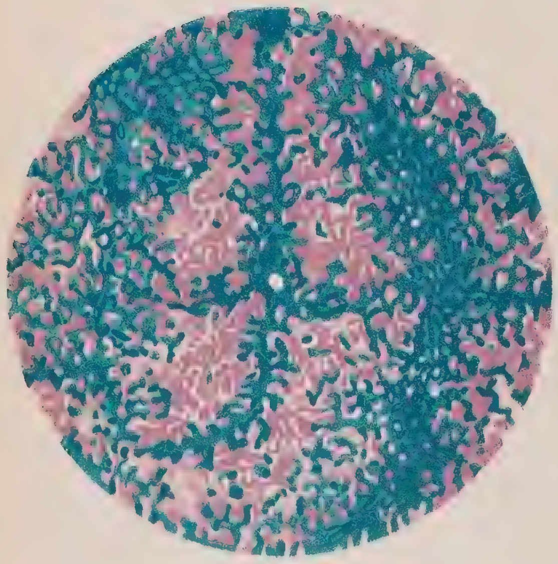

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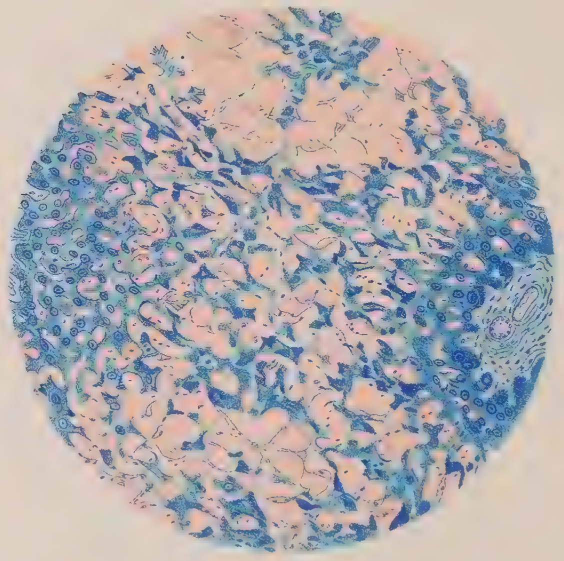

severe cases the liver tissue is of a yellow, yellowish green (Icterus viridis) or green black (Icterus melas) color.

In the interlobular connective tissue dilated gall ducts filled with bile may be seen.

The microscopic examination is best made by the aid of double knife sections or with hardened sections stained with carmine. M. F., F. M.

Lower magnification discloses cells and groups of cells containing green coloring matter distributed as fine lines and spots in the protoplasm. Toward the center of the lobule these cells are grouped together to such an extent that the central area appears distinctly green. (Plate II., Fig. 4.)

Under higher magnification the cause of the green discoloration of the tissue is found in widely dilated bile ducts filled with inspissated bile. The ducts form a network surrounding the liver cells between them and the capillaries. Here and there masses of bile, or distorted cylinders mark the excessive engorgement of the capillaries. In the protoplasm of the epithelial cells minute cylinders of green material may be seen. These are commonly branching and are the injected secretion ducts, or intracellular bile capillaries of the liver cells. (Plate II., Fig. 2.)

In many cells only a faint suggestion of the intracellular capillaries may be observed, while in those near the central veins, especially when the process is advanced, large plates and fragments of green material may be seen filling the cell protoplasm. In advanced icterus granular and crystalline bile coloring matter is not uncommonly deposited in the hepatic cells and the stellate cells of Kupffer. The color of this pigment is yellowish red and it contains no iron. In fresh double knife sections the protoplasm of the cells presents a diffuse yellow coloration.

4. Pigment Infiltration of the Liver.

This process differs from brown atrophy of the liver in the nature of the pigment which is deposited, in this case being hæmatogenous pigment. Deposi-

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DESCRIPTION OF PLATE II.

Fig. 1. Brown atrophy of the liver. See p. 82. High power. Fresh scraping. A group of adjacent liver cells still in contact. Protoplasm contains groups of yellowish brown granules and colorless spherical, and oval fat droplets. The highly refractive nature of the fat droplets is shown by their wide dark borders. The nuclei of the cells are not visible.

Fig. 2. Icterus of the liver. See p. 83. High power. Alum carmine. The liver cells contain a number of green branching and fusiform masses of inspissated bile which fill the secretion ducts within the cell protoplasm and the fine gall capillaries between the cells.

Fig. 3. Pigmentation of the connective tissue cells of the liver. See p. 84. High power. Alum carmine. Groups of yellow pigment between the liver cells. These are spindle-shaped or branching and nuclei may be made out within them. The pigment deposits are in the stellate cells of Kupffer.

Fig. 4. Icterus of the liver. See p. 83. Low power. Alum carmine. The stasis of bile is localized around the central vein. The dark green masses fill the capillaries about the liver cells and extend into the cell protoplasm.

Fig. 5. Amyloid liver. See p. 87. Middle power. Fresh double knife section, treated with Lugol's solution. The amyloid is stained brown. Liver parenchyma yellow.

Fig. 6. Amyloid liver. See p. 87. Middle power. Methylene-violet reaction. Amyloid rose red. Liver parenchyma blue green. (A similar reaction is obtained with methyl-green.)

Fig. 7. Amyloid liver. See p. 87. Middle power. Permanent preparation from hardened material. Embedded in paraffine. Mounted in balsam. Stained with polychrome methylene-blue. (Schmorl.) Amyloid pale rose color. Nuclei deep blue. Cell protoplasm lighter blue.

tion of hæmatogenous pigment in the liver occurs as the result of various blood diseases (pernicious anæmia), following the absorption of repeated hemorrhage, or as a part of a condition of general hæmatogenous pigmentation known as hæmachromatosis. Many forms of cirrhosis of the liver are associated with excessive pigmentation.

Whether the pigment is transported through the blood and deposited in the cells or converted from the blood and taken up by the cells of the liver is a matter of doubt. The condition bears the name of hæmosiderosis, a word which naturally suggests that with appropriate chemicals, the pigment gives a reaction showing the presence of iron.

In hæmachromatosis both iron-containing and iron-free pigment is found. A special form of pigmentation of the liver is associated with the malarial infection. The macroscopic appearances of a liver affected with pigment infiltration, aside from cirrhosis when present, are extremely varied. The color may be any shade between yellow and deep red or brown and often resembles the color of iron rust. As the connective tissue of the liver is often involved, the demarkation of the lobules may be indistinct. The microscopic characteristics are likewise variable.

Fresh examination of double knife sections, or formaline, F. M., carmine. Test for iron (p. 56).

The deposition of hæmatogenous pigment begins in the peripheries of the lobules, and the periportal connective tissue, and extends toward the centers. In the early stages of the infiltration, the localization of the pigment serves to distinguish this condition from that of brown atrophy, where the pigment is first deposited at the centers of the lobules. The periportal connective tissue is not uncommonly alone infiltrated with pigment and occasionally the stellate cells (Kupffer) contain deposits of pigment. (Plate II., Fig. 3.) The epithelial cells of the liver in such cases may be entirely free from pigmentation, or may show beginning atrophic pigmentation at the centers of the lobules (brown atrophy).

As the pigmentation increases in extent the parenchyma becomes infiltrated, the cells at the peripheries of the lobules being first affected, until, in excessive cases, every liver cell contains pigment.

5. **Fatty Infiltration.**

The presence of fat within the liver cells characterizes the most frequent change to which the organ is subject. A moderate amount of fat may be deposited within the liver cells when large amounts of fatty food have been ingested, and the deposition is then a physiological process. After long-continued fevers, or certain chronic disorders, as alcoholism or tuberculosis, the liver is almost invariably the seat of a more or less intense fatty change. Whether the fat is exclusively carried to the cells through the circulation (fatty infiltration) or produced within the cell at the expense of the protoplasm (fatty degeneration) is an unsolved problem. Undoubtedly the former is the most common process.

Where the process is not far advanced the deposit of fat is confined to the peripheries of the lobules, and may be seen when the organ is cut, as a delicate yellow tracery. Where the deposits of fat do not include the entire periphery of the lobules, the deposits are localized about the periportal areas, producing an interrupted network. The periportal areas are thus surrounded by a yellow zone which renders it a matter of some difficulty to distinguish this condition

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from that in which the fat is deposited in the center of the lobules, the so-called central fat infiltration.

As the fat deposit increases the network becomes less transparent and of whitish yellow appearance. Where cyanosis of the organ is present in combination with fatty infiltration, the dark red centers of the lobules stand out in marked contrast and give the organ an appearance not unlike that of a nutmeg (known as "nutmeg liver"). In cases of extensive fat infiltration, not associated with cyanosis, as in alcoholism, the cut surface of the enlarged liver may present a uniform yellow appearance and the tissue is more or less opaque, cloudy and brittle.

The microscopic investigation is best begun by scraping the cut surface of the liver and examining the fluid obtained.

In spite of the varied character of the macroscopic appearances of the tissue in the various forms of fat infiltration and degeneration, there is one element, when examined microscopically, which is common to all of these conditions; the fat-containing liver cells. (Plate II., Fig. 1.) The distribution of the fat in the liver cell differs from fat deposits in other cells, by the great difference in the size of the fat droplets. In the heart and skeletal muscles, the fat is deposited in the form of minute drops, having a more or less definite arrangement. The fat deposited in the liver cell takes the form of large drops of varying size, which fill out the protoplasm of the cell and not uncommonly displace the nucleus which, with the remaining protoplasm, may be pressed against the periphery of the cell in the form of a crescent. Spherical form, shiny appearance and double contour distinguish the fat droplets. Their characteristic appearance is unchanged on the *addition of acetic acid or potassium hydrate.* In the act of scraping the surface of the liver a large number of cells are broken up, thus liberating numerous fat droplets which swim free in the fluid. The free fat droplets must be distinguished from minute air bubbles which are commonly found in fresh preparations, by noting the position of the bright spot on focussing. If the bright spot lies above the object and appears on focussing upward (when the microscope tube is being raised), we are dealing with fat. If the bright spot appears on focussing downward, the object is an air bell.

Double knife sections examined by low power show a dark zone at the peripheries of the lobules and, when cyanosis is present, red centers. On the addition of acetic acid or potassium hydrate, the periportal connective tissue and the center of the lobule clear, leaving the peripheral fat zone opaque.

On examination with high power the presence of liver cells containing pigment may be demonstrated at the centers of the lobules, a condition produced by the cyanotic changes in the organ.

In hardened sections (*M. F., F. M., N. S.*) the appearance is somewhat different, as the embedding or mounting process has removed the fat, leaving large vacuoles in the protoplasm. (Plate III., Fig. 2.) Here again may be seen the various forms of fat-containing cells, and the peripheral distribution of the fat. In the affected zone the liver structure is nearly destroyed, only the centers of the lobules showing the characteristic network of liver cells. Fat may be preserved in embedded tissue by hardening it in an osmic acid mixture, such as Flemming's or Hermann's solution. The fat is stained black.

In rare cases the fat is deposited in the cells at the center of the lobules. Such a specimen is shown in Plate IV., Fig. 1. Equally rare is a form of fat infiltration in which the fat droplets are deposited in the stellate cells of Kupffer. In both of these forms the fat droplets are very small and resemble the minute droplets seen in fatty degeneration of the heart muscle.

The so-called acute yellow atrophy of the liver is a destructive process which affects the entire organ, and leads to a true fatty degeneration of the protoplasm. In this case the fat is formed from the albuminous substances of the protoplasm. The cause of the disease is either severe infections or intoxications, such as phosphorus poisoning. The degeneration is so intense that the cells are entirely destroyed, freeing the fat drops which are later absorbed, leaving only the vascular connective tissue framework of the liver, which presents a deep red appearance. During the progress of the disease the liver, which may be enlarged (early stage) or remarkably decreased in size (late stage), presents a variegated appearance, the yellow areas of fatty degeneration intermingling with the more degenerate and deeply red portions of the tissue.

The amount of contrast which the affected areas present depends upon the degree to which the degeneration of the tissue has advanced ; in fact, the appearance of the tissue may vary as widely as there are degrees of fatty degeneration.

Microscopically the conditions are the same. Areas in which the liver cells show fatty infiltration lie adjacent to groups of lobules in which the cells are affected by true fatty degeneration, and in others the liver cells are completely disintegrated, only detritus remaining. The red portions of the tissue contain capillaries filled with blood, isolated patches of liver cells, Kupffer's stellate cells, and pigment deposited from the bile or blood in the form of reddish yellow rhomboid plates, or fine needles. The periportal connective tissue is infiltrated with round cells which indicate the inflammatory nature of the process.

Beside these marked evidences of destructive change when the process is advanced, areas may be found in which the liver cells show evidence of more or less successful attempts at regeneration. Such areas may appear macroscopically as small yellowish points standing out against the background of liver tissue.

Microscopically they appear as enlarged liver lobules, the cells of which are not arranged in the usual form, but in bands many rows in width. The diameters of the cells are much greater than those of the normal epithelium of the liver.

A second form of regeneration may be found in marked branching and new growth of the bile capillaries.

These develop in the neighborhood of the red areas in the periportal connective tissue, and consist of fine canals, often accompanied by isolated liver cells.

These canals are continuations of the normal bile capillaries and are generally derived from them. In some cases the new gall ducts end in areas of liver cells which appear to form a continuation of the canal, and are strongly suggestive of a transformation of gall duct epithelium into the specific epithelium of the liver.

When the organ affected by acute yellow atrophy is exposed for a period of time, a whitish deposit forms upon the cut surface. When examined microscopically this deposit is composed of crystals of leucin and tyrosin.

The first of these, leucin, appears in the form of brownish spheres, occasionally with concentric striation. Close examination reveals a fine radiating structure. Occasionally they may resemble fat but are not soluble in ether. Tyrosin appears in the form of fine needles arranged in bundles or sheafs.

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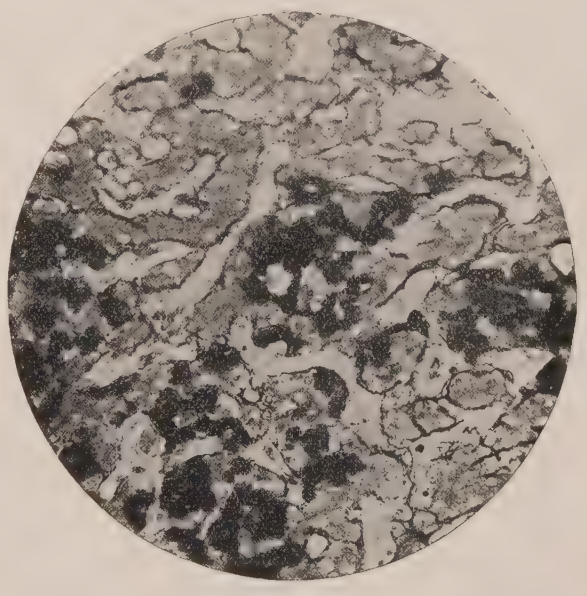
6. Amyloid Degeneration of the Liver.

Amyloid deposits in the liver occur as part of a general process affecting various organs (liver, spleen, kidney, intestine, etc.), but occasionally the process is limited to one organ. It is most commonly observed in the organs of individuals who have been subject for a long period to purulent disease of bones or joints, tuberculosis or syphilis. The peculiar transparent appearance of organs in which the process is advanced is especially well seen when a thin slice of the tissue is held against the light. Beside this, the organ is heavy, stiff and inelastic. These changes in the macroscopic appearance of the organ are due to the deposition within the tissue of a peculiar, glassy, transparent, albuminoid product—amyloid. On the cut surface of the organ the amyloid change may be instantly detected when a solution of iodine (Lugol's solution) is poured upon the tissue, whereupon the amyloid material turns a deep brown. (Plate II., Fig. 5.) As the alkaline reaction of the tissue interferes with the reaction, it is best to acidify the surface by first pouring a dilute solution of acetic acid upon it. The distribution of the amyloid substance in the liver is variable. Often the iodine reaction produces small isolated spots through the tissue, occasionally a fine network appears, and again all the lobules appear as brown dots, while in rarer cases the periportal connective tissue is alone affected. Great variety is found in the microscopic appearances.

FIG. 13.



Amyloid degeneration of liver. Mallory's connective tissue stain. Dark masses amyloid. The connective tissue stroma surrounding the hepatic cells is sharply defined.

The examination of the tissue may be made by the aid of fresh sections cut on the freezing microtome. A short period of hardening in Müller's fluid or formaline gives excellent results. Material which has been hardened for a longer period of time, loses the characteristic staining reactions of the amyloid. If fresh, unstained sections of the tissue are examined by high power, the presence of large numbers of colorless, highly refractive masses may be made out. These form a definite structure and occupy a distribution in the lobule about half way between the center and periphery. The shining material lies between the liver cells which are atrophic and compressed in the portions of the tissue where the deposit is the greatest. If, in addition to the amyloid change, other retrograde alterations of the tissue are present, as deposits of pigment in the center of the lobule and fatty infiltration at the periphery, we then have a complex picture in which we may distinguish within the lobule an inner yellow, a middle glistening, and an outer dark zone. The shiny mass deposited in the intermediary zone consists of amyloid which may be shown when the tissue is treated with iodine or gentian-violet solutions. (Plate II., Fig. 5.) (See technique, page 50.) When treated with iodine the amyloid material takes on a brown color which changes to blue on the addition of sulphuric acid, for which reason the material is known as amyloid, meaning, like amylum-starch. When treated with gentian-violet solution the amyloid material is stained red, the surrounding parenchyma bluish, the nuclei of the cells deep blue. (Plate II., Fig. 6.) Permanent preparations may be made with polychrome methylene-blue, which stains the amyloid rose colored, protoplasm blue, nuclei deep blue (Plate II., Fig. 7) and Mallory's connective tissue stain, which stains the liver stroma deep blue, parenchyma red, amyloid lighter blue. (Fig. 13.)

Careful examination of hardened material with high power will demonstrate that the amyloid is located between the endothelium of the capillaries and the liver cells, which suggests that the amyloid is deposited from the blood within or around the vessel walls.

The greater the amount of amyloid the greater is the pressure upon the liver cells and the atrophy resulting therefrom. (Plate III., Fig. 3.) The capillaries are not alone affected, as we have seen in the first examination with low power. The larger vessels, those lying within the periportal areas, are likewise the seat of amyloid deposit and are surrounded by a wide brown or red ring, as the case may be.

Higher power shows the amyloid material to be deposited principally within the vessel walls, the greatest change being in the media, where the amyloid is deposited between and separates the muscle fibers.

7. Abscess of the Liver.

Abscess formation is a common sequel to invasion of the liver by bacteria, amœbæ and other parasites. These find entrance to the organ through the hepatic arteries (ordinary general sepsis), through the portal vein (infection of the lower portion of the intestinal tract, *i. e.*, dysentery) and through the gall ducts and lymph vessels. The greater the number of organisms carried to the organ, the greater the number of abscesses disseminated through the hepatic substance. Beginning abscesses appear in the form of minute yellow dots, sprinkled over the cut surface of the organ. Where the avenue of infection has been the gall ducts, the almost invariably accompanying stasis of bile, which is the cause of the infection in some cases, gives the abscesses a yellowish green color.

When the infection is fresh, the presence and location of the organisms is best demonstrated in fresh double knife sections, or sections cut with the section razor from tissue hardened in alcohol.

The application of dilute acetic acid to the section clears the surrounding tissue, causing nuclei, pigment (in fresh sections, likewise fat), and the masses of organisms, all of which are unaffected by the reagent, to stand out distinctly. The organisms appear as dark masses, which are resolved into minute spherical bodies, or rods, the uniformity in size or characteristic form serving to distinguish them from fat droplets. These collections of organisms in the capillaries, or gall ducts, form the foci of irritation about which develop the abscesses. The growth of the abscess is due to the migration from the blood vessels in the immediate neighborhood, of polynuclear leucocytes, which collect about the source of irritation (in this case the organisms).

F., F. M. Stain with Lœffler's methylene-blue or Gram, or, for amœbæ, with Mallory's thionin stain. The first stain is to be used where the organisms have

PLATE III.

Fig. 1.

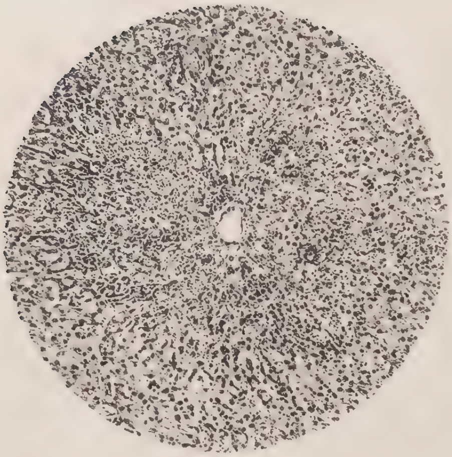


Fig. 2.

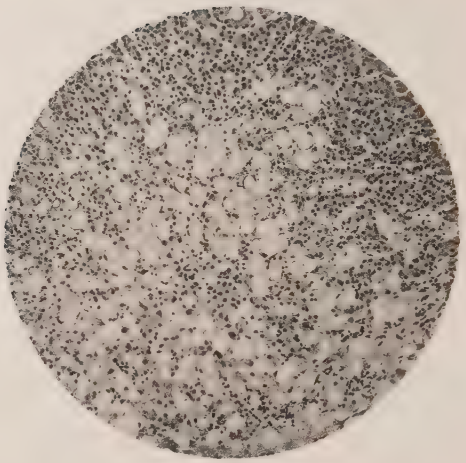


Fig. 3.

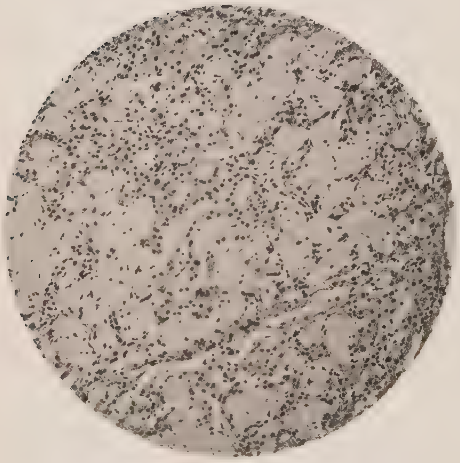


Fig. 4.

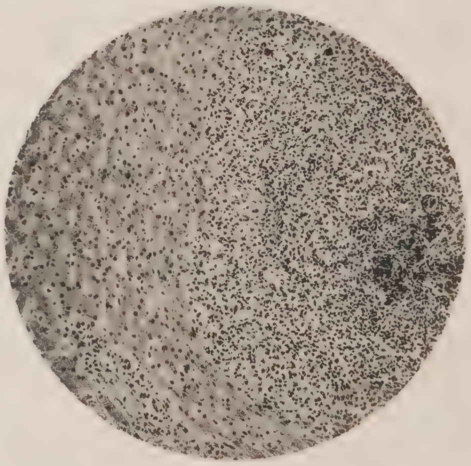


Fig. 5.

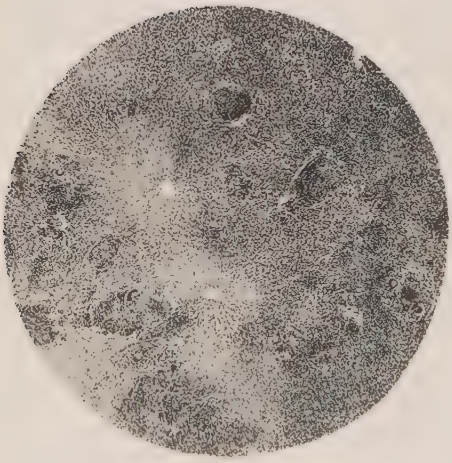
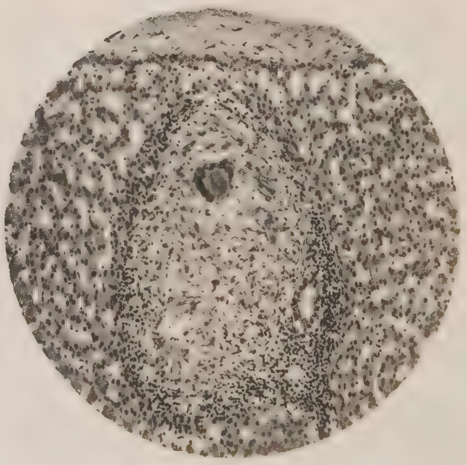


Fig. 6.



found their way to the liver through the gall ducts, as in such cases the infectant is usually the bacillus coli communis, which is decolorized by Gram's method. Where the abscess is due to amœbæ coli it is advisable to employ all three methods; the thionin for the differentiation of the amœbæ from the cells (the nuclei of the amœbæ staining red, those of the tissue blue), methylene-blue for the demonstration of the colon bacillus, and Gram for the demonstration of any accompanying pyogenic organisms (staphylococci and streptococci).

By low power the abscesses may be seen as blue areas in the tissue within which small, deeply (by Gram, blue) stained spots may be demonstrated. The smaller abscesses may be located near the periportal areas, or centers of the lobules, according to the method of invasion. Larger abscesses ordinarily involve many lobules or even a large part of the organ.

The before-mentioned green color of abscesses is seen in hardened sections to be due to masses of inspissated bile. The larger the abscess the more plainly the following characteristics may be observed: An unstained center in which are seen small, deeply stained clumps; surrounding the unstained center, a wide, deeply stained zone of leucocytes; at the extreme periphery of this zone an area of hemorrhagic infiltration.

The smaller abscesses, when examined with higher powers, show the greater part of the abscess to consist of polynuclear leucocytes, which are massed at the center and which, at the periphery, divide up into more isolated groups. The deeply stained masses at the centers of the larger foci are found to consist of organisms which, if the infection is fresh, are lodged in the capillaries or gall

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DESCRIPTION OF PLATE III.

Fig. 1. Cyanotic atrophy of the liver. See p. 81. Middle power. Hæmatoxylin. At the center a central vein. At the extreme periphery the well-preserved liver cells with deeply stained nuclei. The capillaries between them are widely dilated. Toward the center the liver cells gradually become atrophic and disappear (*magnifier*). The widely dilated capillaries contain a large number of deeply stained bodies, blood corpuscles (*magnifier*).

Fig. 2. Fatty infiltration of the liver. See p. 85. Middle power. Hæmatoxylin. The characteristic structure of the liver lobule is obliterated. At the upper portion of the figure the characteristic appearance of the liver cells may be noted. Below and at the center in place of the liver cells a large number of round openings. These represent drops of fat which have been removed during the embedding process.

Fig. 3. Amyloid degeneration of the liver. See p. 87. Middle power. Hæmatoxylin. At the extreme right of the figure the edge of a central vein. The arrangement of the liver cells is more or less characteristic. At the center and to the left liver cells widely separated, the spaces between being filled with the highly refractive homogeneous, unstained, amyloid material.

Fig. 4. Edge of liver abscess. See p. 89. Middle power. The abscess (to the right) is composed of closely packed leucocytes the nuclei of which may be seen with the *magnifier*. The dark masses at the center of the abscess are masses of deeply stained organisms. The liver cells at the periphery of the abscess show the characteristic concentric flattening which occurs about rapidly developing abscesses or tumors.

Fig. 5. Miliary tuberculosis, liver. See p. 94. Very low power. Near the center two central veins in cross section. These form the centers of two lobules about the periphery of which are darkly stained groups of cells (tubercles). When examined with the *magnifier* these (especially that to the right) contain dark crescentric bodies (giant cells). At the center of the tubercle on the right, beginning caseation.

Fig. 6. Miliary tubercle, liver. See p. 94. High power. The tubercle lies directly beneath the capsule of Glisson. The center of the tubercle is composed of reticulated connective tissue (*magnifier*) and contains large giant cells. About the periphery of the tubercle a well-defined round celled infiltration (*magnifier*).

ducts. Later the intense infiltration of leucocytes, which causes a dissolution of the tissue, obliterates all landmarks within the abscess. The tissue adjacent to the septic emboli shows marked necrosis.

As the abscess extends the action of the organisms and their products upon the leucocytes becomes apparent. This is seen in massing and separation of the chromatin within the nuclei, and is followed by disintegration of the protoplasm, with the liberation of free masses of chromatin. As a result of this action the centers of the larger abscesses, with the exception of the masses of bacteria or amœbæ, are unstained; then comes an area marked by deeply stained granules of free chromatin, outside of which is a wide zone of well-preserved leucocytes, surrounded by the zone of hemorrhagic infiltration. A characteristic concentric flattening of the adjacent liver cells, due to pressure, accompanies the larger abscesses. (Plate III., Fig. 4.)

When the liver is exposed to the action of toxic substances, the tissue is commonly sprinkled with minute foci of necrosis (so-called focal necroses). This condition occurs in exaggerated cases of icterus following closure of the bile ducts and in severe intestinal infections, but is seen in most typical form in puerperal eclampsia (Schmorl). Macroscopically these small focal necroses have a dull yellow appearance and in severe cases the tissue may be thickly studded with them.

F., F. M., N. S. or formaline alcohol. F. M., Weigert's fibrin stain.

The tissue in the necrotic areas when seen under the microscope shows absence of nuclear stain. The blood capillaries in the necrotic tissue are filled with homogeneous masses which are apparently continuous in places with the disintegrated liver cells. Where the tissue has been stained for fibrin the entire network of capillaries in the necrotic area and the necrotic tissue itself is found to be permeated with a network of fibrin.

8. Cirrhosis of the Liver.

The term "cirrhosis" includes a great variety of chronic inflammatory processes affecting the connective tissue of the liver. These changes may lead either to enlargement of the liver (hypertrophic cirrhosis), or to a great diminution in the size of the organ with hardening of the tissue (atrophic cirrhosis—cirrhotic induration). The condition of cirrhosis may be recognized from that of ordinary atrophy (brown or simple cyanotic atrophy) by the appearance of the surface of the organ which shows marked irregularities and a distinct nodulated character (granular atrophy). The substance of the organ, when incised, presents a similar appearance and the cut surface, shortly after section, presents the same nodular characteristics as the parietal surface. The nodular projections of the free or cut surface present great variation in color, ranging from intense yellow to reddish yellow, red or even green.

Because of the yellow coloration of the tissue Laennec gave to the condition the name "cirrhosis" (from *κιῤῥὸς*, yellow). On examination of the parietal or cut surface it may be readily determined that these nodules of varying size include larger or smaller collections of liver lobules. These projecting islands of liver tissue are surrounded and isolated by a grayish white network of dense connective tissue which is exceedingly prone to contract and, on section of the liver, causes the protrusion of the liver lobules above the surface, giving it the nodular appearance above described. Fresh double knife sections treated with

Reference:

Schmorl. Unters. über Puerperaleklampsie, 1893, Leipzig. For changes in the liver in typhoid fever see Mallory, The Journal of Experim. Medicine, Vol. III., 1898.

acetic acid and examined under the microscope suffice for preliminary examination of the tissue.

In such sections the groups of liver lobules appear darker than the surrounding tissue and occasionally have a distinct yellowish shimmer. These islands of hepatic tissue are separated by wide, clear bands and spaces. Under high power the islands of liver tissue are resolved into characteristic liver cells, the majority of which contain deposits of fat droplets and pigment, and occasionally between them may be seen green clumps of inspissated bile. In the clear portions of the tissue, now seen to be composed of connective tissue, are found numerous blood vessels and gall ducts. The finer relations of the tissue must be studied in sections of hardened and embedded material. F., F. M., carmine, Van Gieson or elastic fiber stain, or Zenker, embedded in colloidin. Mallory's connective tissue stain.

In the early stages of cirrhosis the structure of the liver lobules and the liver tissue show no change even when examined with low power. Parenchymatous degenerations (fatty degeneration) which are believed by some observers to be the cause of the proliferation of the connective tissue, may be present, but in a certain number of cases even these changes may not be detected.

The first indication of cirrhosis is the appearance of round cells and an increase of spindle cells in the periportal connective tissue (Plate IV., Fig. 3). These changes, as we have already observed in the consideration of other organs, indicate a progressive inflammatory change of the connective tissue stroma. The production of round cells and the increase of fixed spindle cells proceed gradually, the process beginning in the areas directly about the blood vessels, spreading along the interlobular stroma and leading ultimately to the production of wide bands of cicatricial tissue surrounding and isolating the lobules. When the process is in a state of progression, this fact is marked by the presence of round cells, which are found even when the production of connective tissue is advanced, and disappear only when the process has ceased to be active.

If the sections be stained suitably the connective tissue is found to contain a great number of newly formed elastic fibers.

The natural result of this increase of the stroma is a gradual separation and isolation of areas of tissue which, owing to the unequal distribution of the process, more often consist of groups (multilobular) than of isolated lobules (monolobular or annular form). The increase of connective tissue seldom confines itself to the trabeculæ dividing the lobules (interlobular), but projections force their way into the lobules (intralobular), splitting them up and disturbing the intralobular arrangement of the cells. All types of the process may be found in the same organ, so that a classification of cirrhosis on this basis is scarcely practicable.

The examination of the isolated groups of liver lobules fails to disclose the typical arrangement of the liver cells within them. The original well-defined network and radiating structure is entirely destroyed. The proliferation of the connective tissue into the lobules and between the cells has totally obliterated the characteristic appearance. The newly formed capillaries may be successfully injected from the hepatic artery, but with great difficulty from the portal vein. This derangement of the capillary circulation causes obstruction to the flow of blood in the portal system and leads to the development of ascites, one of the classical symptoms of cirrhosis of the liver. Naturally, a marked increase in the connective tissue exerts direct pressure upon the liver cells, from which these become atrophic, pass through various stages of degeneration and ultimately perish. The continuity of the network of gall capillaries is thus seriously interfered with, with the result that in many portions of the liver there is retention of bile and the production of icterus.

Further changes are found in the bands of connective tissue. Even lower powers show within the increased fibrous structure parallel rows of deeply stained nuclei (Plate IV., Fig. 4), which in advanced cirrhosis intercommunicate, often presenting a net-like arrangement, in some cases covering an area of the size of a normal liver lobule. The arrangement of these net-like structures is in some cases highly suggestive of the liver structure, although the cells are much smaller, but higher magnification shows the parallel rows to consist of cubical epithelium, which have small, spherical, deeply-stained nuclei.

Because of the low cubical nature of the epithelium lining these canals, they might possibly be mistaken for blood capillaries, in which, however, the epithelium is much flatter and the nuclei are more widely separated. As normally but a few gall ducts are found in the periportal connective tissue, and as these usually invade the liver lobule for a short distance, we must conclude that these newly formed canals represent a proliferation of the gall ducts. That proliferation is actually in progress may be proven by the presence of numerous karyokinetic figures in the nuclei of the cells.

Whether these ducts are the result of proliferation of the original bile ducts or consist of atrophic liver epithelium, for which explanation the net-like arrangement and the apparent transition at the periphery of the lobules of liver cells into the cubical epithelium of the ducts are suggestive, are questions which cannot in all cases be decided. Beside these structures, various compensatory changes in the liver epithelium may be noted, especially increase in size of many cells which often contain more than one nucleus.

The distribution of these hypertrophic forms is suggestive, being most commonly found near the periphery of the lobule, and sometimes directly in apposition to the beginning of one or more gall ducts passing into the connective tissue stroma. At such points the before-mentioned forms suggestive of a transition of liver cells into the epithelium of the gall ducts are more commonly encountered.

Where a sufficient number of these groups of hypertrophic liver lobules are present, the condition is spoken of as nodular hypertrophy of the liver. They

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DESCRIPTION OF PLATE IV.

Fig. 1. Fat deposit at the center of a liver lobule. See p. 85. Low power osmic acid preparation. At the center a central vein. To the right and above a periportal area. In the liver cells about the central vein a large number of minute, spherical bodies, *i. e.*, fat droplets blackened by the osmic acid. Toward the periphery these become fewer in number. Extreme periphery of the lobule practically free.

Fig. 2. Leucæmia of the liver. See p. 96. Middle power. Hæmatoxylin. At the center a central vein. The nuclei of the liver cells are poorly stained. Between the network of liver cells the capillaries are filled with closely packed leucocytes the nuclei of which are deeply stained. (*Magnifier.*)

Fig. 3. Fresh cirrhosis of the liver. See p. 91. Middle power. Hæmatoxylin. At the center a periportal connective tissue area surrounded by adjacent lobules. Within this area cross sections of gall ducts and vessels. The connective tissue about these is thickly infiltrated with round cells. The liver tissue to the right shows evidence of fatty infiltration.

Fig. 4. Advanced cirrhosis of the liver. See p. 91. Middle power. Hæmatoxylin. The liver lobules are separated by wide bands of connective tissue which may be detected by their light color. In the connective tissue above and below is seen a well-developed, round-celled infiltration. To the left and above the round-celled infiltration invades the liver parenchyma (*magnifier*). At the extreme periphery above cross section of two gall ducts and scattered through the wide bands of connective tissue a large number of gall ducts in cross and oblique section. These may be recognized by the single row of closely arranged cubical epithelium with spherical nuclei.

PLATE IV.

Fig. 1.

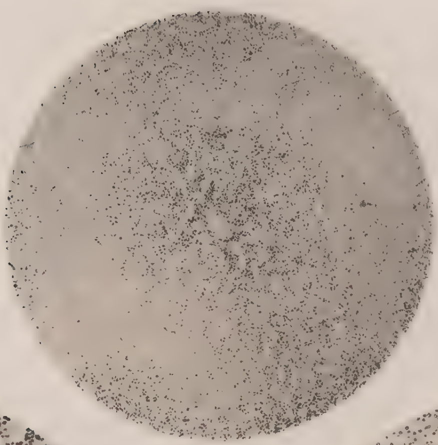


Fig. 2.

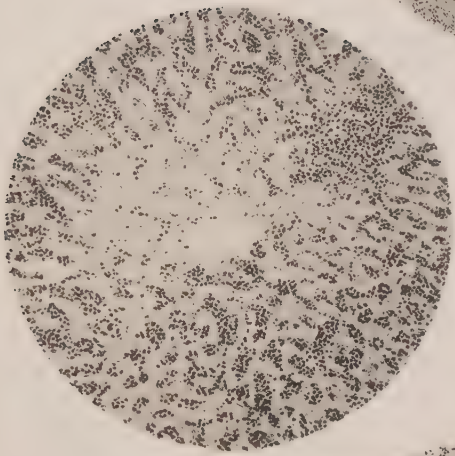


Fig. 3.

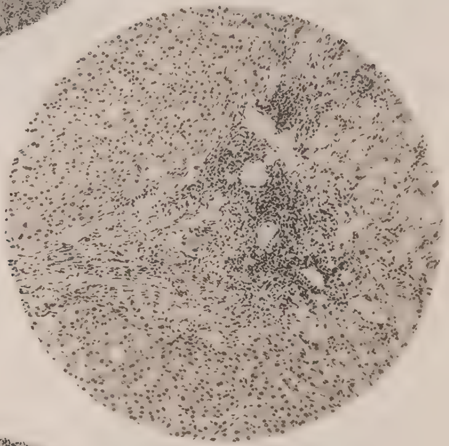
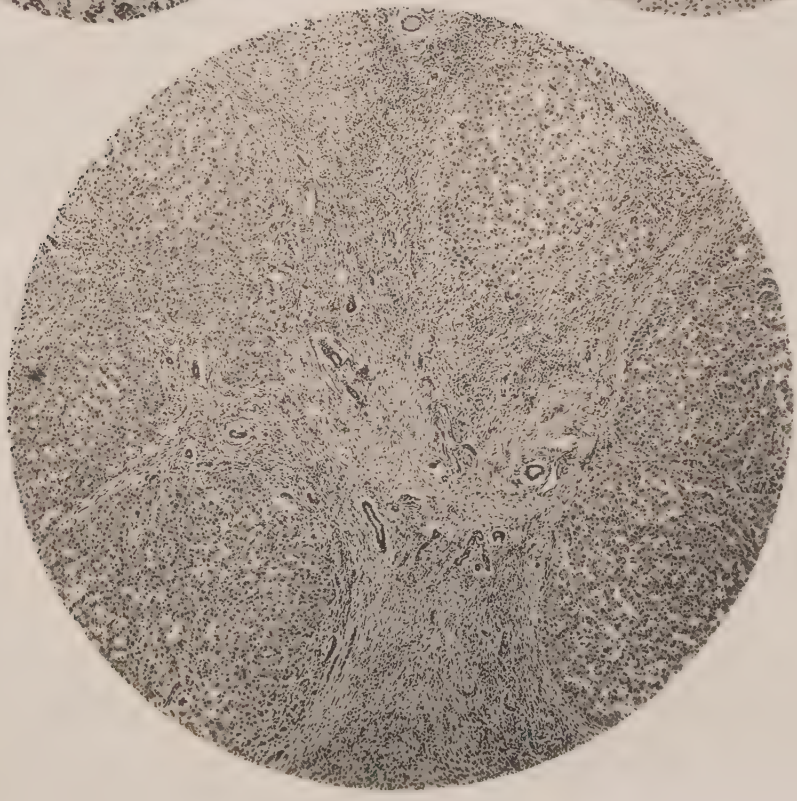


Fig. 4.



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appear on the free and cut surface of the liver as tumor-like projections of the surface and of paler color than the surrounding tissue. A complete series of transition stages exists between these hypertrophic areas and the remaining degenerated parenchyma.

Compensatory hypertrophy of portions of the hepatic tissue is characteristic of cirrhosis, but it is likewise found wherever large portions of the parenchyma are destroyed, as in acute yellow atrophy, cyanotic atrophy and in the neighborhood of echinococcus cysts or tumors. As to the cause of cirrhosis little is known. The action of various toxic substances as alcohol, lead, toxic substances from the intestinal tract and a variety of infections (intestinal infection, malaria, syphilis, etc.), have each been credited with the production of various forms of cirrhosis. According to the nature of the irritant or the manner in which it reaches the liver, the character of the process may differ. Thus the irritant may reach the liver by way of the blood vessels, by way of the gall ducts, or through the peritoneal covering of the organ. As the process advances a differentiation of the various forms becomes more difficult. Beside ordinary cirrhosis of the liver (Laennec) a special form of hypertrophic cirrhosis, leading later to atrophic cirrhosis and combined with icterus and enlargement of the spleen, but not associated with ascites, has been described. Even the ordinary cirrhoses have been divided into a perivascular and a biliary form, according as the principal changes are localized about the blood vessels or the gall ducts. In the diagnosis of cirrhosis of the liver, the macroscopical relations, clinical picture and the condition of the gall ducts must all be considered. The microscopic examination alone does not always enable us to distinguish between the various forms of cirrhotic change.

In specific hypertrophic cirrhosis (described by Ackermann as elephantiasic cirrhosis) the connective tissue is said to show excessive proliferation and particularly great intralobular development. In biliary cirrhosis the accumulation of bile in the tissue produces numerous focal necroses about which develop proliferations of organizing connective tissue which may be considered separately from the general proliferative process in the organ. Compensatory hypertrophy of certain lobules reaches its greatest development in the biliary form of the affection.

9. Tuberculosis of the Liver.

The most frequent form is that associated with general miliary tuberculosis, when the liver structure is filled with minute tubercles. The so-called solitary tubercle which takes the form of large tubercular areas, sometimes as large as a hazelnut, is much rarer. In both cases the tubercles show the characteristic grayish white periphery, with the yellowish opaque center. In the miliary and submiliary form the tubercles are generally numerous and may, by the unaided vision, be detected lying directly beneath the capsule of Glisson, or in the cut surface of the organ. They appear as minute grayish white dots, occasionally with the yellow centers indicating caseation, or of a greenish color indicative of a close relation between the tubercle and the bile ducts. In very early stages of the infection the presence of tubercles may be first demonstrated under the microscope.

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Greco. Über die Reproduktion der Leber bei Lebercirrhose. Riforma Medica, Vol. III., Nr. 22.

Kretz. Über Lebercirrhose. Wiener klin. Wochenschrift, 1900, Nr. 12.

An excellent idea of the number and size of the miliary and submiliary tubercles may be obtained by examining with low power a fresh double knife section treated with acetic acid.

The triangular periportal connective tissue areas appear as clear spaces between the dark lobules. Adjacent to these are clear oval or circular areas, likewise more transparent than the lobular tissue, and measuring from a quarter to a third of the diameter of a lobule. These are tubercles which usually include one or more giant cells. A more accurate understanding of the structure of the tubercle is obtained by studying hardened sections.

F., F. M., N. S., or tubercle bacilli stain, or formaline alcohol, F. M., or embed. Weigert's fibrin stain.

In stained sections the tubercles appear as deeply stained spots (low power) frequently arranged near the periphery, where, when numerous, they may form more or less complete circles surrounding the lobules. (Plate III., Fig. 5.) Within the tubercles (high power) closer examination shows the presence of areas which are uniformly stained (by carmine unstained), within which no cells may be demonstrated. These are areas of disintegrated and necrotic tissue represented by the macroscopically visible yellow spots. In general the tubercles present the structural arrangement already described.

The more acute the character of the disease and the fresher the tubercles the greater will be the predominance of the round cells. The more chronic the process the greater the number of epitheloidal cells, these being in all probability derived from the fixed connective tissue or endothelium of the lymph spaces (epitheloidal tubercle). The round cells form a peripheral zone surrounding the epitheloidal cells. In very chronic processes the epitheloidal elements exhibit a tendency to the production of connective tissue. This may consist of a fibrous structure within the tubercle (reticulated tubercle) (Plate III., Fig. 6), or lead to the development of distinct fibrous connective tissue.

Occasionally a form of spontaneous cure results from the transformation of the epitheloidal cells into connective tissue, producing a sort of capsule about the tubercle and impeding its growth. This newly formed connective tissue usually falls a victim to the caseation which begins in the center of the tubercle and invades the surrounding tissue. The presence of giant cells and the absence of blood vessels distinguish the tubercles. In fresh cases the presence of fibrin may be demonstrated within the tubercle.

The epitheloidal form of tubercle is that most commonly found in the liver. In Plate III., Fig. 6, the epitheloidal character of the cells may be demonstrated. Here the groundwork of the tubercle shows a reticulum. Caseation is not present, but may be seen in another tubercle by examining Plate III., Fig. 5, with a magnifying glass. For the demonstration of tubercle bacilli the well-known specific staining methods are employed.

10. Syphilis of the Liver.

Syphilis appears in the liver in two forms, congenital and acquired. Congenital syphilis begins in the structures about the umbilical vein, through which the infection is transferred from the mother to the fœtus, whence it is immediately conveyed to the liver. The changes produced are exceedingly variable, cirrhosis and miliary gummata predominating.

Syphilitic cirrhosis is of the progressive, intralobular type. The lobules are broken up, the radiating arrangement of the cells is destroyed, and the islands

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Zehden. Zus. Referat. Centr. f. path. Anat., Bd. 8, 1897.

of liver cells consist of isolated groups of irregularly formed and arranged masses of protoplasm, many of which contain multiple nuclei. These changes in young children commonly lead to imperfect development of the organ. Miliary gummata, when these appear, take the form of nodes of intimately intermingled round and spindle cells. The gummata are usually localized about the periportal connective tissue. When the centers of the gummata are necrotic, they present an appearance not unlike tubercles, with the exception that giant cells are rarely present. Miliary gummata in the new-born must not be confused with the accumulations of cells which surround the dilated capillaries of the organ, the so-called proliferation centers which represent the last effort of the liver in the production of red and white blood corpuscles, a function which that organ exerts during the latter part of fœtal life.

Syphilis of the liver in the new-born is not uncommonly associated with excessive icterus (brimstone liver). Lastly, a form of congenital syphilis of the liver exists, in which only a moderate number of isolated gummata are found, and these are localized about the umbilical vein, and are associated with inflammatory proliferation in the wall of the vein and general or localized cirrhosis of the liver. Gummata are the characteristic product of syphilitic infection of the liver, and are found in somewhat larger form in the acquired disease, which shall alone receive our attention, owing to the great rarity of the congenital form.

The appearance of the syphilitic liver is characterized by deep cicatricial depressions, usually located at or near the fissure of the suspensory ligament (hepar lobatum). An incision made perpendicular to the surface of one of these depressions exposes a pyramidal area, near the apex of which may be seen spots of varying size, somewhat yellowish in appearance and very suggestive of caseated tubercles. These are gummata. There are, however, characteristic differences between these nodules and tubercles of similar size, although the difference may not at the first glance be demonstrable. The gumma is surrounded by a more or less well-defined zone of connective tissue, giving it a distinct whitish periphery. Often the yellowish nodule is buried in a wide area of scar tissue. The cicatricial character of the surrounding connective tissue permits it to contract after section, producing a distinct prominence of the yellowish center, a relation seldom found in tubercle. Ordinarily gummata are larger than tubercles, which seldom exceed the size of peas or hazelnuts.

A possible difficulty might arise in distinguishing gummata from the large nodular form of tuberculosis. The localization of the nodules about the region of the cicatricial depressions will serve to distinguish gummata from tubercles which develop in any region of the liver.

Examination of a fresh double knife section made through a gumma immediately discloses the presence of a large amount of fat in the necrotic material, and especially in the peripheral proliferating zone. In tuberculous caseated material and in tuberculous granulation tissue, fatty degeneration plays but an unimportant rôle, or may be entirely absent, although occasional exceptions to this rule may be met. A general distinction may be made between the character of the necrotic process in syphilis and tuberculosis. In syphilis the necrotic process is associated with fatty degeneration and in tuberculosis the necrosis is usually accompanied by hyaline degeneration. Formaline, F. M., N. S., or stain for elastic fibers or harden in Flemming or Hermann, for which purpose select small pieces of tissue from the periphery of the gumma.

In hardened sections the areas of necrosis are characterized by the absence of nuclear stain. The greater the nodule the more widespread the caseation and necrosis. The structure is most characteristic in small gummata. (Plate V.,

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M. B. Schmidt. Ziegler's Beiträge, Bd. XI.

Fig. 2.) As in some forms of tubercle, the smaller gummata may contain a moderate amount of connective tissue growth. The diagnosis of gumma is greatly assisted by the investigation of the blood vessels. The walls of the smaller vessels show marked alteration early in the syphilitic process. This consists of a marked thickening of the media and adventitia, changes which are not found to the same degree in tuberculosis. The changes in the vessels precede and are often the beginning of the gumma, within which may frequently be recognized the remains of the altered vessels. The poison of syphilis, the nature of which is not definitely determined, is no doubt present in the blood, as the alteration of the vessels suggests. Distinct epitheloidal and giant cells, so characteristic of tuberculosis, are seldom present in gummata. The periphery shows a distinct zone of long spindle cells extending toward the center. Surrounding the zone of spindle cells are numerous round cells, and numerous polynuclear leucocytes. Giant cells are very rare in gummata. The adjacent liver structure shows the effects of pressure (concentric flattening, and brown atrophy).

Far beyond the region of cicatrix formation characteristic cirrhotic changes appear in the periportal connective tissue of the liver. Not uncommonly the entire organ presents a picture of advanced cirrhosis or granular atrophy. The cicatricial tissue surrounding the gummata is rich in elastic fibers. Plate V., Fig. 1, shows the periphery of a gumma of the liver which has been hardened in Flemming's solution to better demonstrate the deposit of fat. The black dots scattered through the necrotic area are fat droplets and in the wide band of cicatricial tissue surrounding the central necrotic area may be seen accumulations of large fat drops. Within the cicatricial zone an obliterated vessel may likewise be seen. The adjoining liver tissue shows marked fatty changes.

11. Leucæmia of the Liver.

Leucæmia is best defined as a condition of increased activity (probably due to irritation) of the organs concerned in the production of the corpuscular elements of the blood. Lowit, a recognized authority on the blood has recently described protozoa in the blood of leucæmic patients which he considers the cause of the disease. The disease may principally affect one organ, as the red marrow of bone (myelogenetic leucæmia), the spleen (lienatic leucæmia), or the lymph nodes (lymphatic leucæmia). The result of the irritation of these organs is an increase of the specific elements which, on passing into the blood, cause an increase of the various forms of leucocytes. As the disease progresses other organs, especially the large glandular organs of the abdomen, present characteristic changes. In the liver, accumulations of leucocytes are found about the vessels and in the periportal tissues. Whether these accumulations consist of cells which have wandered out of the blood vessels, or whether they are due to a proliferation of preëxisting lymphatic rests, which are occasionally found in the normal organ, remains to be determined. The macroscopic examination reveals an enlarged liver.

On section the periportal areas are enlarged and prominent, presenting a general appearance not unlike cirrhosis. Under the microscope (low power) the first glance suggests a beginning cirrhosis; that is, the periportal tissue is filled with cells. High powers show these to be leucocytes, and the liver lobules present radial striation produced by more or less complete filling of the capillary spaces with leucocytes. (Plate IV., Fig. 2.) *M. F., F. M., N. S.*

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The large vessels likewise show an enormous increase of leucocytes, these points rendering a differentiation between leucæmia and cirrhosis of the liver a matter of small difficulty. The character of the leucocytes varies with the different forms of the disease. Usually they are of the small or large mononuclear type.

In rare cases, usually in typhoid fever, lymphoid tissue may be found in the periportal connective tissue without corresponding changes in the blood. These nodes commonly possess a necrotic center, but are free from giant cells. If the tissue be stained with methylene-blue, groups of faintly stained typhoid bacilli may be demonstrated about the necrotic center. In pseudo-leucæmia, which is a disease of the blood-forming organs without the increase in the number of cellular elements of the blood which accompanies leucæmia, similar lymphomata may be found in the periportal connective tissue of the liver. The lymphoid accumulations in pseudo-leucæmia differ in no way from those of true leucæmia.

12. Hydatid Disease of the Liver.

(*Echinococcus.*)

The tænia echinococcus, an intestinal parasite of the dog, is a small tapeworm about 4 mm. long, divided into three segments the last of which produces large numbers of ova. The eggs of the tænia are occasionally found about the mouth and in the hair of the infected animal, and may from thence be transferred to man. Once in the alimentary tract of man, the developing embryo penetrates the intestinal wall and is deposited in the larger organs, notably the liver. Here it develops a connective tissue capsule and, after a period (weeks or months) of growth, may be encountered as a vesicular structure, with a laminated membrane, approximating in size a walnut. (Echinococcus cyst.)

The cyst wall is composed of two layers, the outer laminated structure being known as the cuticular and the inner granular as the parenchymatous layer. From the inner layer are developed the so-called budding or brood capsules. As time passes, the cyst, which contains a clear fluid, rich in sodium chloride and free from albumin, increases in size, often to that of a child's head, or larger. From the fourth month the development of the so-called brood capsules begins. These commence as minute elevations of granular appearance on the interior surface of the primary cyst, and develop into small secondary cysts, upon the surface of which may be detected depressions which mark the development of the scolices. The scolex measures about 0.3 mm. in length, and consists of two segments, one of which is attached at its base to the wall of the brood capsule. The free end of the scolex is crowned by a circle of hooklets, directly beneath which, upon the sides of the shorter segment, are arranged four suckers with cup-like depressions in their centers. The longer segment of the scolex, being that part attached at the base, often contains glistening chalk granules. The scolices become detached from the cyst wall, and through bursting of the brood capsule are found swimming free in the larger or parent cyst. Should an organ affected by echinococci be eaten by a dog, the scolices develop within the intestinal tract of the animal to mature tænia echinococci. A common occurrence within the main cyst is the development of secondary cysts (daughter cysts) from the primary membrane, and these becoming detached swim free within the primary cyst cavity. Of greater rarity in man, but common in animals, is a form in which the daughter cyst develops without the primary membrane and produces multiple cysts. (Echinococcus veterinorium.) Naturally the development of these enormous cysts produces widespread atrophy of the surrounding tissue. The membrane of the parent cyst becomes ultimately surrounded by a secondary capsule of connective tissue. If, as occasionally happens, the scolices perish, the wall of the cyst becomes shrunken and impregnated with lime salts, and loses its characteristic structure.

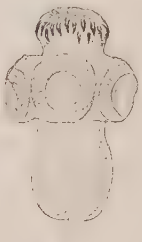
In fresh cases where the cyst is still growing, the diagnosis is usually easy, although large cysts of a similar appearance occur in the liver and a certain diagnosis can only be made with the aid of the microscope. *An examination both of the cyst wall and of its contents* must be made. The material for the investigation is best obtained by cutting small cross sections from the soft cyst wall with a pair of shears. As the cyst wall proper is often several millimeters thick, and may be readily stripped from the more dense connective tissue capsule, these cross sections of tissue may be cut without difficulty. The characteristic appearance of the cyst wall may be determined by low power, better still by high power, when it is found to possess a distinct concentric lamination. (Fig. 14.)

Here and there upon the inner surface of the membrane may be seen granular masses of parenchyma, from which the brood capsules are developed. Scissor sections made from the walls of the daughter cysts show the same concentric lamination found in the wall of the large cyst. If the inner surface of the echinococcus cyst be carefully examined it is found to be covered with numberless fine white projections about half the size of a pinhead. If these are scraped from the surface and placed under the microscope they are found to consist of brood capsules, in which the already described scolices are found. Many of the brood capsules have burst for which reason the fluid within the cyst usually contains numerous scolices. An examination of the free scolices shows with great distinctness the suckers and crown of hooklets. (Fig. 15.) The highly refractive granules of the body of the scolex are found to disappear on the addition of acetic or hydrochloric acid. They consist of chalk. Where no brood capsules are found in the principal cyst, they should be sought for in the daughter cysts. If one of these be held against the light, the brood capsules may usually be detected as dark granules, either attached to the cyst wall or swimming free in the clear or milky fluid of the cyst. If the cyst be carefully opened and its contents collected, the free brood capsules may be removed without difficulty, by filtering the fluid. Brood capsules and scolices may not always be demonstrable in the echinococcus cyst as they not uncommonly perish or are destroyed. Unfortunately this is always the case where the cyst is calcified and the characteristic lamination of the cyst wall is

Fig. 14.

Laminated wall of an echinococcus cyst (fresh).

Fig. 15.



Extruded scolex from a brood capsule.

obliterated. A diagnosis in such cases could not be made were it not that the hooklets of the scolices usually resist the degenerative process. Therefore, the contents of the suspected cyst should be carefully examined for the presence of hooklets. This is best accomplished by centrifuging the contents of the cyst and carefully examining the sediment. A similar examination must be made of all suspicious calcified nodes in the liver, first by decalcifying the tissue with hydrochloric acid, then making a careful search for hooklets. The hooklets of the echinococcus scolex are much smaller than those of other tapeworm larvæ, measuring but 0.04 mm. Where the calcification of the degenerating echinococcus cyst is not too excessive, the mushy contents of the sac are found to contain large amounts of fat and cholesterin plates. Investigation of the connective tissue capsule is best made by the aid of sections from hardened material. These should include the cyst wall, the surrounding capsule and the adjacent liver tissue. Harden in formaline, F. M., or embed in paraffine or celloidin, N. S., or elastic tissue stain.

The connective tissue capsule is found under low power to consist of fibrous tissue not unlike that developed in the liver in advanced cirrhosis. Here and there areas of round-celled infiltration are found, and many newly formed gall ducts. The same questions as to whether the newly formed gall ducts represent an attempted regeneration, or a purely inflammatory proliferation may be again raised in this condition, as we have already indicated in cirrhosis. Scattered through the connective tissue capsule may be found accumulations of giant cells. These are not identical in appearance with those found in tuberculosis, where the nuclei are commonly arranged at or near the periphery of the cell. These giant cells contain multiple nuclei which are usually gathered together in a group at the center. The application of specific staining methods for the demonstration of bacilli within these giant cells gives negative results. These are the so-called foreign body giant cells, which develop in the neighborhood of foreign bodies which are embedded in the tissue, and especially in the neighborhood of parasites which have perished. They probably play a part in the general disintegration and absorption of the intruding body.

IV. THE KIDNEY.

Structure.—The kidney is composed of a series of uniform structures, each representing in itself an independent kidney system called a reniculus. We distinguish in these separate renal systems, as well as in the kidney itself, a general division into two parts, that portion lying next the periphery of the kidney being known as the cortex, and the portion adjacent to the pelvis being called the medulla. The medulla consists of a series of pyramids, the apices of which project into the pelvis of the kidney, forming the papillæ.

The cortex is divided into two portions known as the medullary rays (pyramids of Ferrein) and the substantia glomerulosa, or labyrinth. This last is so called because of its characteristic arrangement, which consists of tufts of capillaries which project into and are surrounded by open spaces lined with epithelium. These receive the fluid secreted from the tufts of vessels, or glomeruli and are lined with distinct capsules, which cover the surfaces of the blood vessels as well as the inner wall of the free spaces and are known as the capsules of Bowman.

Beginning at a point opposite the glomerulus is a tube, through which the secretion escapes. This tube for a short distance describes a very irregular course, and is known as the convoluted tubule. The epithelium lining it is continuous with the epithelium of the capsule of Bowman. At the point where the convoluted portion ends the tube descends in a straight line for some distance, often into the cortical portion of the kidney, then bends upon itself and returns in a straight line, thus forming a loop with a descending and an ascending arm, which is known as the loop of Henle.

The lumen of the descending portion of the loop is lined with flattened epithelium which protrudes inward with its convex surface toward the lumen and presents an appearance very suggestive of the endothelium lining ordinary capillaries. The lumen of the ascending portion is supplied with a single layer of cubical cylindrical epithelium. The point of transition from the flattened epithelium of the descending arm to the cubical epithelium of the ascending arm is usually located slightly above the turn of the loop, in the descending arm. The ascending arm terminates in a second convoluted or connecting portion, which is shorter than the first convoluted portion and enters directly into the straight tubes forming the medullary portion of the kidney. The first convoluted portion of the tube and the thick arm of the loop of Henle exercise the specific secretory function of the organ. From these portions of the tubes are excreted the solid substances of the urine. The connecting or second convoluted portion appears to exercise more an absorbing than a secreting function. The watery constituents of the urine are excreted through the glomeruli. The protoplasm of the cylindrical epithelium of the convoluted tubule when examined in the fresh state is found to be finely granular in character. These granules are scattered through the protoplasm but in the basal portion of the cell are grouped together in rod-like masses which are placed transversely to the long axis of the cell. The free surface of the epithelium is covered with fine cilia. These cilia may be demonstrated only in very fresh material, as they are rapidly destroyed by post-mortem change. The tubuli contorti, the connecting portions, the glomeruli and the supplying blood vessels constitute the principal portion of the substantia glomerulosa or cortex. Each segment of cortex which lies between two groups of medullary rays is supplied by a single interlobular artery, the fine divisions of which supply directly the capillaries of the glomeruli. The medulla is composed for the greater part of the loops of Henle and the straight connecting tubes which form the pyramids. The collecting tubes unite at the apex of the papillæ where they form the so-called ductus papillaris. The epithelium of the uriniferous tubules in their entire length rests upon a structureless basement membrane or tunica propria.

DESCRIPTION OF PLATE V.

FIG. 1. Edge of a liver gumma. Advanced stadium. See p. 96. Very low power. Osmic acid. Safranin. Above and slightly to the left, necrosis. Below this and forming the central portion of the figure a wide area of connective tissue containing deeply-stained dots (fat-containing cells). This wide band of connective tissue is the so-called connective tissue capsule of the gumma. To the right in cross section a small vessel with thickened wall. To the left of this a delicate oval outline within which is a mass of connective tissue, the remains of an obliterated vein (*magnifier*). Beneath the adjacent liver lobules and central veins. Marked fatty deposits in the cells at the periphery of the lobules.

FIG. 2. Periphery of a liver gumma. See p. 96. Middle power. Early stadium. Hæmatoxylin. Above necrosis surrounded by a zone of granulation tissue thickly infiltrated with lymphocytes and leucocytes. Beneath characteristic structure of the liver (*magnifier*).

FIG. 3. Parenchymatous nephritis. (Cloudy swelling.) See p. 101. Very high dry power. Hæmatoxylin, neutral red. The epithelial cells markedly swollen, the protoplasm finely granular, the nuclei poorly stained, in many cells entirely absent (necrosis), the boundaries between the cells poorly defined.

FIG. 4. Fatty degeneration of the kidney. See p. 102. High power. Osmic acid. Safranin. To the left a glomerulus. To the right convoluted tubules. The epithelial cells of the tubules contain near their bases a large number of closely packed fat droplets (*magnifier*). These are blackened by the action of the osmic acid. Here and there in the glomerulus may be found an occasional fat droplet.

FIG. 5. Fresh septic infarct kidney. See p. 105. Very low power. Hæmatoxylin. To the left deeply stained and well-preserved kidney parenchyma. To the right pale necrotic tissue of the infarct. Below the center, a partially occluded vessel, the tissue adjacent to which is more or less perfectly stained. At the surface of the kidney (above) the infarct is covered by a narrow margin of deeply stained tissue which derives its nourishment from the vessels of the capsule.

FIG. 6. Edge of a fresh infarct of the kidney. See p. 104. Middle power. Hæmatoxylin. To the left living deeply stained tissue. To the right a necrotic area. Embedded in the necrotic area a glomerulus, which is transformed into an indefinite hyaline mass (*magnifier*). The infarcted tissue is infiltrated with leucocytes. The nuclei of the epithelium of the tubules are partly preserved, in most cases broken down into fine granules (karyorhexis).

PLATE V.

Fig. 1.

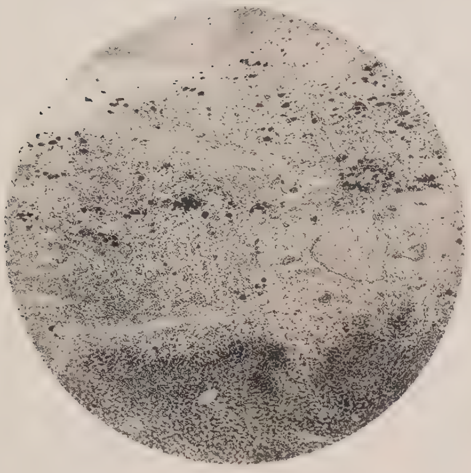


Fig. 2.

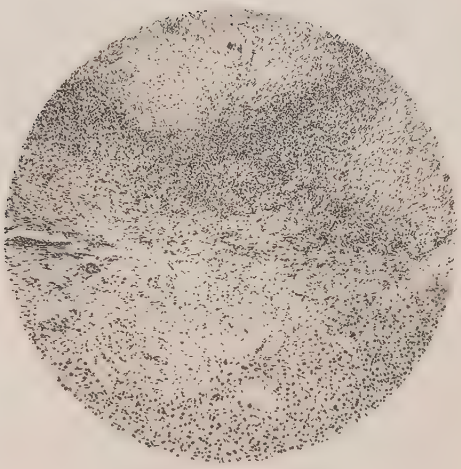


Fig. 3.

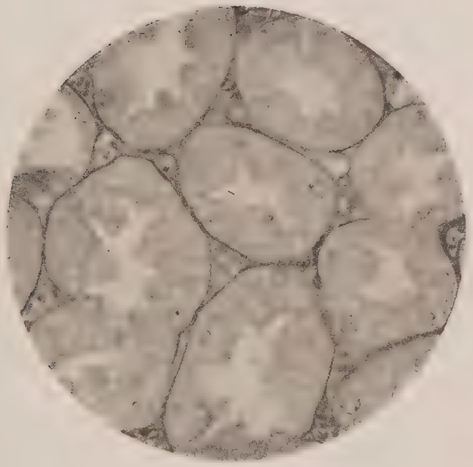


Fig. 4.

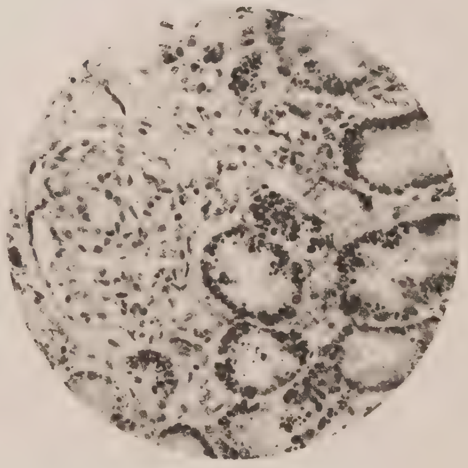


Fig. 5.

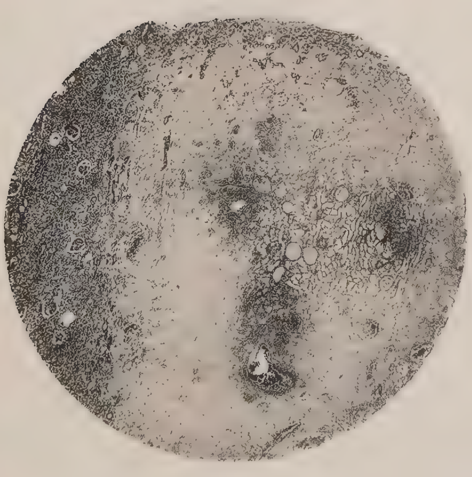
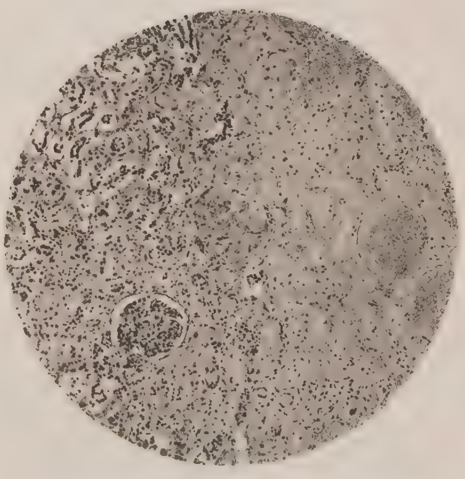


Fig. 6.



Gaylord & Aschoff — Pathological Histology

The contrast between the cortex and medulla in a normal kidney may be plainly seen on section. The cortex is of reddish gray color, while the medulla, owing to its greater vascularity, is of a deep red. Extending from the medulla into the cortex may be seen the pyramids of Ferrein, also of a deep red color. To the parenchyma (composed of the epithelium lining the tubules and the capsule of Bowman) and the blood vessels must be added the connective tissue structure which holds them together. The amount of connective tissue in the kidney is, in contrast to most glandular structures, very small. In fact, it is often difficult to demonstrate, except in the areas surrounding the larger blood vessels, which are to be found at the junction of cortex and medulla. It is most easily seen in transverse sections of the uriniferous tubules. Elastic tissue is very rare in the kidney and is only found in the neighborhood of the blood vessels.

A microscopic examination of the kidney, whether made upon fresh or hardened sections, should always be conducted with the following points in mind: first, the condition of the parenchyma; second, the condition of the glomeruli; third, the condition of the blood vessels; fourth, the condition of the connective tissue (including elastic tissue).

Only by observing the condition of these various constituents of the kidney can a correct diagnosis be made, as it is possible for but one of the various component structures of the kidney, for instance the glomeruli, to be affected. Ordinarily, sections cut perpendicular to the surface of the kidney, including cortex and medulla, are sufficient. Where, however, interstitial changes are suspected, horizontal sections should likewise be made.

1. Parenchymatous Nephritis.

In acute infectious disease, poisoning, etc., the kidney is enlarged, the cortex in particular being swollen, and on section projecting above the medulla. The color contrast is also more marked, the cortex being more gray than in the normal organ and the medulla intensely red. Aside from being paler than normal, the tissue of the cortex has become more or less opaque. This is most apparent when the observer notes the difficulty with which vessels lying directly beneath the surface are detected. In the normal organ the cortex is sufficiently transparent to allow of a considerable penetration of vision into the superficial layers. In parenchymatous nephritis this quality disappears, the tissue being so altered that light is directly reflected from the surface, hence the opaque appearance. The cause of this change may be well observed in simple scrapings from the cut surface of the cortex examined in salt solution. A preparation from a normal kidney shows nests and short cylinders of renal epithelium. From the affected kidney, however, we find largely isolated cells and cell detritus. The cells are swollen and are darker than normal, and where they adjoin the cell boundaries are indistinct. Under high power the protoplasm is found to be filled with granules of varying size, many of which are unusually large. On the addition of acetic acid these granules disappear, and in many cells the nuclei become visible. Formaline, F. M., N. S., or van Gieson or harden in Altmann's mixture, granula stain, or formaline alcohol, F. M., or embed. Weigert's fibrin stain or van Gieson.

These changes may be well observed in hardened sections (M. F., F. M., N. S.) when embedded in glycerine. In preparations embedded in balsam the granulæ when not specifically fixed or stained (van Gieson, Altmann, Weigert) are scarcely visible, owing to the high refractive index of the balsam. The irregular distribution of the change may be well observed in hardened sections. The tubuli contorti and the loops of Henle are most commonly affected, while the collecting tubules are relatively immune. The increase in bulk of the epithelium commonly obliterates the lumena of the tubules, while a tendency to disintegration which affects the superficial portions of the epithelium may produce a false lumen filled with detritus.

Extensive vacuole formation is frequently found in the superficial region of the cell protoplasm. This may be so extensive as to entirely fill the lumen of the tubule. In hardened sections the boundaries of these vacuoles appear in the form of a fine network dividing up the lumen of the tubule. The degenerative processes in the protoplasm are followed by desquamation of the epithelium which most commonly affects the epithelium of the glomeruli. One point of importance which may be observed in hardened sections is the variability with which the nuclei of the cells take on the staining reagent. In certain cells or groups of cells the nuclear stain fails entirely. (Plate V., Fig. 3.) With the destruction of the nucleus the cell is doomed. Parenchymatous degeneration of the cells may therefore lead to total necrosis. The fact that changes in the epithelium similar to those already described may be the result of post-mortem change, must not be overlooked. A positive diagnosis of parenchymatous degeneration is only possible when the tissue is examined shortly after death.

2. Fatty Degeneration.

Like parenchymatous nephritis, fatty degeneration is the result of many acute and chronic infectious diseases, poisoning, anæmia, diabetes, etc. The macroscopic appearance of the kidney varies with the severity and the distribution of the change. When the process is advanced the organ is enlarged and stiff. The most characteristic change is a distinct yellow coloration of the tissue. This may often be detected on the surface of the kidney, but is more prominent on cut surfaces, where the yellowish cortex, which is often alone affected, stands out in contrast to the red medulla. Careful examination resolves the general yellow color of the tissue into minute comma and S-shaped figures, which are confined to the substantia glomerulosa. Occasionally the medullary rays alone are affected, in which case the change is marked by minute, parallel, yellow lines extending into the medulla. In other cases the kidney structure has a yellowish mottled appearance, not confined to any especial structure. As suggested by the variety of macroscopic appearances, the microscopic examination is full of interest. Fresh double knife sections treated with acetic acid or potassium hydrate give the best results.

Treated with acetic acid and examined with low magnification, the opaque section clears, and the irregular distribution of the fat becomes more apparent. We observe the characteristic localization of the fat droplets in the epithelium of the tubuli contorti, and we may easily demonstrate that the minute comma and S-shaped figures in the tissue, observed with the naked eye, are identical with the affected tubules. Preparations treated with acetic acid reveal plainly the nuclei and the structure of the tissue, while in preparations treated with potassium hydrate, everything disappears except the fat, by which means the local character of the change is even more clearly shown.

Higher magnification shows the cell protoplasm to be filled with fat droplets of varying size, which in the early stages of the process are arranged near the base of the cell. (Plate V., Fig. 4.) The connecting portion of the tubule is that most commonly affected. When the macroscopic examination reveals a fine yellow striation of the medullary rays, the epithelium of the straight tubules and of the loops of Henle will be found affected. When the macroscopic appearance is a general mottling of the kidney, the fat will be found to occupy the spaces between the tubules, that is, in the connective tissue of the kidney. High magnification shows the accumulations of fat droplets arranged in spindle or branching forms, in conformation with the structure of the connective tissue cells.

The description of the above forms of fatty degeneration does not exhaust the list of variations of this change in the kidney. In many cases the glomeruli are the seat of the degeneration. Microscopically the fat will be found to occupy the covering epithelium of the glomerulus. Fresh double knife or freezing microtome sections may be stained with Sudan III. when the fat droplets appear a brilliant red.

Reference has been made to the varying size of the fat droplets. In cases where they are very fine and more uniform in size it is possible to mistake them for chalk granules or cocci, the differential diagnosis of which will be discussed later.

The finer distribution of the fat is best observed in specimens hardened in osmic acid. Thin pieces of tissue are hardened in Flemming's mixture, when, after thorough washing, they may be embedded or transferred to formaline 10 per cent., 12 hours, and cut on the freezing microtome, or the formaline may be omitted and the tissue embedded. The fat takes on an intense black. (Plate V., Fig. 4.)

3. Anæmic Infarct.

Should a vessel of sufficient size in any organ become occluded, especially through closure of its lumen by an embolus, the result is that the section of tissue which it supplies, which is usually pyramidal in shape, having its apex near the vessel and its base at the periphery of the organ, becomes anæmic. This anæmia causes a pale, whitish appearance of the tissue. In certain organs, especially the lungs, the anæmic area, under special conditions (chronic congestion), becomes inundated with blood, which, flowing from the capillaries, fills the tissue; hence the name (red hemorrhagic) infarct, a term which is inappropriately applied to the anæmic areas above described, and which are also known as infarcts.

The anæmic area may be detected on inspection of the surface of the kidney, as an irregular pale area or spot, surrounded by a red zone. If the infarct is fresh it usually projects above the surface. When it is old it is usually depressed. On section the tissue is found to be pale and dry and is likewise surrounded by the red zone. Ordinarily the infarct lies in the cortex but not uncommonly it sends one or more projections into the intermediate zone between the medulla and cortex. It is in this zone that the larger vessels of the kidney are placed and when the incision of the tissue has been fortunately placed, one may detect the occluded vessel which has led to the formation of the infarct. Owing to the distribution of the vessels and the structure of the cortex the anæmic areas in the kidney usually have a cubical form. (Ribbert.)

Fresh infarcts, including the side lying parallel to the surface of the organ, are usually surrounded by a well-defined red (hyperæmic) zone. In older infarcts a narrow, yellowish zone may be demonstrated within the red zone, thus giving a double contour. The examination *of a double knife section* is sufficient to explain the above-described phenomenon. The red zone will be found to consist of an intense hemorrhagic infiltration, while between it and the infarct proper will be found an area in which the tissue shows marked fatty degeneration, and which corresponds to the yellow zone. The infarct proper will be found to consist of

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Hausemann. Über das vorkommen von Fettinfiltration der Niere. Virchow's Archiv, 148, 1897.

necrotic tissue or degenerate masses with merely a suggestion of the original tissue structure. In hardened sections (F., F. M., N. S. or F. A., F. M., Weigert's fibrin stain) the same conditions may be seen, with the exception that the fat has disappeared, owing to the embedding process.

The infarct proper consists of an unstained area, in which occasional migrating leucocytes appear. The structure of the tubules and glomeruli may usually be made out, but the protoplasm of the cells has lost its structure and is reduced to a homogeneous or granular mass. (Plate V., Fig. 6.) These are the remains of the albuminoid products of the cell protoplasm, which, under the influence of the lymph circulation, have become converted into hyaline substance (so-called coagulation necrosis. Weigert). The necrosis of the tissue is accompanied by coagulation of the fibrin in the adjacent blood vessels, and capillaries of the glomeruli with occasional exudation into the uriniferous tubules. To determine the amount of fibrin formation, a specific fibrin stain must be employed. The boundaries of the infarct are distinctly delimited against the surrounding tissue. At the periphery, that portion of the tissue which corresponds to the red zone in the gross specimen, the capillaries, especially those of the glomeruli, are markedly engorged. In many cases the walls of the capillaries are broken through and the blood flows into the surrounding tissue (hyperæmic and hemorrhagic peripheral zone). These appearances indicate the energy with which the blood in the surrounding tissue endeavors to flow into the necrotic area through the vessels. These, however, owing to the coagulation necrosis, which necessarily includes the walls and contents of the vessels, have become occluded. Within the hyperæmic zone is found a narrow margin of tissue in which large numbers of deeply stained nuclei are found. Under high power these are resolved into leucocytes, which lie between the degenerate uriniferous tubules and which diminish in number as the necrotic tissue is approached. This zone of leucocytic infiltration is not found in all infarcts, neither is it equally well marked in the different stages of infarct formation. Fatty degeneration of the protoplasm of these leucocytes aids, to no small extent, in the production of the yellowish zone of fatty infiltration detected in the fresh, double knife section. At the zone of leucocytic infiltration the necrotic tissue begins but is seldom complete. Here and there single, usually straight, tubules may be found in which the nuclei take the stain. The epithelium of these tubules, when examined in the fresh state, shows marked fatty degeneration. The degeneration of the leucocytes and of such epithelium as is not totally necrosed combine to produce the yellowish zone which separates the zone of hyperæmia and hemorrhage from the necrotic center of the infarct. The fatty changes in the partially necrotic epithelium indicate a diminished and insufficient metabolism, which is sufficient to support the life of the cell, but which is not capable of preventing degenerative changes in the protoplasm (so-called necrobiosis).

Directly beneath the capsule of the kidney, and separating it from the base of the infarct, will be found a narrow band of kidney structure in which the nuclear stain is preserved. This area is supplied by branches from the arteries of the capsule, which are independent of the arteries of the kidney substance. (Plate V., Fig. 5.) The fresher the infarct the more likely are we to find areas of tissue which are still preserved, especially around the blood vessels and the glomeruli. We may also expect to find in the necrotic tissue an occasiona isolated glomerulus, with well-stained nuclei.

The first reaction of the epithelium to disturbed metabolism and approaching necrosis is a characteristic clumping or precipitation of the chromatin (karyorhexis). In the center of the infarct, where the removal of the chromatin does not occur so rapidly as in the hyperæmic periphery the necrotic tissue is commonly filled with these granules. As the process advances the coagulation necrosis affects even the remaining glomeruli and the boundary between living and dead tissue becomes indistinct. In place of a hyperæmic zone a margin of vascular granulation tissue composed of round and spindle-shaped cells develops and penetrates more or less widely into the necrotic tissue. The remains of the hyperæmic zone may be found in occasional deposits of granular crystalline blood pigment. In the course of time the entire necrotic area is replaced by young connective tissue (organization). Like all cicatricial tissue, this gradually contracts, leaving a depressed cicatrix to mark the site of the previous infarct. In the neighborhood of old infarcts, the adjacent uriniferous tubules give evidence of more or less successful attempts at regeneration in the form of projections of epithelium beginning with the tubules and penetrating more or less deeply into the site of the infarct. Similar attempts at regeneration may be found in the neighborhood of fresh infarcts, where occasionally the nuclei of the epithelium of the tubules which have escaped the general destruction, show the most beautiful karyokinetic figures. The newly formed cells attempt to replace the necrotic elements by pushing themselves between the old cell and the tunica propria and by this means the lumen of the tubule may ultimately receive a new lining of epithelium. In some cases a complete ring of living cells may be found surrounding the desquamated mass of necrotic epithelium which occupies the center of the tubule. The conditions which favor this regeneration of the epithelium have not been determined.

4. Septic Infarct.

So long as the embolus which occludes the vessel is non-infective, we speak of a benign embolus and a common or simple infarct. Should the embolus consist of infective material a septic infarct results. Macroscopically the change is seldom perceptible, although careful examination will show the yellowish zone to be of a purulent character, and the hyperæmic zone to be much broader than in the simple infarct. Harden in F., F. M. Gram or methylene-blue. The microscopic examination at once shows a marked difference from the simple infarct in a much wider, deeply-stained zone between the hyperæmic zone and the necrotic center. Higher magnification resolves this zone into a dense infiltration of polynuclear leucocytes (pus cells). The hyperæmic zone is broader and more hemorrhagic in character than in the common infarct. In the necrobiotic area we find deeply stained streaks near the blood vessels, which may be resolved into veritable swarms of polynuclear leucocytes, and finally the entire necrotic area is infiltrated with migrating elements.

The leucocytic infiltration about the periphery of a septic infarct does not disappear or diminish as the age of the infarct increases. It remains more or less fixed in extent so long as the action of the bacteria continues. As a result, the long-continued accumulation of leucocytes about the periphery leads to a softening and absorption of the tissue, forming what might be called a line of demarkation about the infarct. When the organisms are stained they are usually found in masses in the embolus or may be its single constituent. Not uncommonly the smaller vessels in the infarct are plugged with masses of

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organisms. The toxic substances elaborated by the organisms, which permeate the tissue are the irritating agents which cause the transmigration of leucocytes and their accumulation about the periphery of the infarct.

5. Renal Abscess.

(*Descending Purulent Nephritis.*)

If by any means large numbers of bacteria find entry into the circulation, a condition of general infection known as "sepsis" is developed. The organisms find lodgment most readily in the lungs, on the heart valves, and in the spleen and kidneys. For this reason whenever a suspicion of acute sepsis is awakened and these organs show no demonstrable macroscopic changes, a careful microscopic investigation of the vessels of these organs should be made for septic emboli.

The microscopic examination may be satisfactorily made by the aid of fresh double knife sections or with free hand sections from pieces of tissue hardened in alcohol. The section is cleared with acetic acid or potassium hydrate. If fatty degeneration is not marked or the specimen has been hardened in alcohol when the fat is abstracted, the masses of cocci may be at once detected as minute dark spots in the clear tissue. It may be necessary to carefully investigate a number of sections before an embolus is found. Higher magnification resolves these masses into minute spheres or rods of uniform size, which may be differentiated from the surrounding fat drops by their lower refractive index. The location of the emboli will be found to be in the intertubular capillaries. In extreme cases the capillaries may be practically injected with organisms. It must, however, be borne in mind that the organisms may have increased in number during the time intervening between death and the autopsy and that the condition found may not accurately express the pathological changes at death. The position of the organisms in the capillaries is best seen in hardened sections. F., F. M., N. S. *or Gram.* Often the capillaries of the glomeruli contain emboli of organisms. (Plate VI., Fig. 1.)

These are the changes most commonly found in early sepsis. Where the septic process is localized within the circulation, as in ulcerative endocarditis, the constant liberation of new masses of organisms may lead to the most widespread plugging of the vessels in the kidney or other organs. This flooding of the capillaries may lead to other changes in the kidney than those described. The organisms may pass through the capillary walls of the glomeruli into the tubules and be carried through the flow of urine to the bladder and thence ejected. Many, however, find lodgment in the tubules and establish lesions similar to those which result from lodgment in the capillaries. (Plate VI., Fig. 1.) If the time is sufficient, whether the organisms be lodged in the vessels or tubules, the focus becomes surrounded by leucocytes and as we have seen in the case of the heart and the liver, an abscess is formed.

To the naked eye the kidney now presents an altered appearance. The cortex and medulla may contain minute yellow spots, which may be round, oval, or in the medulla form minute lines. Often the cortex or medulla is alone affected, and this division indicates the finer localization of the abscesses. Those confined to the cortex are mainly due to occlusion of the capillaries (embolic foci); those found in the medulla are usually located in the tubules (excretion foci). (Plate VI., Fig. 2.) The greater the number of abscesses in the cortex the greater the possibility of organisms passing the glomeruli and becoming

PLATE VI.

Fig. 1.

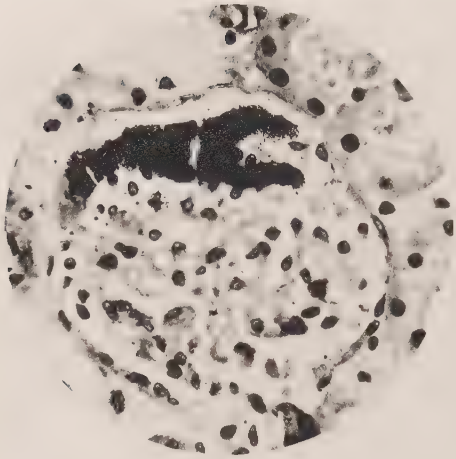


Fig. 2.

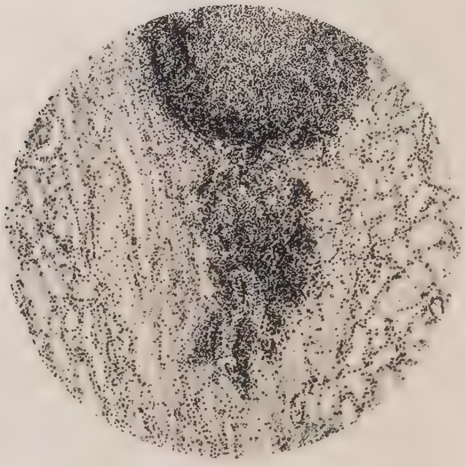


Fig. 3.

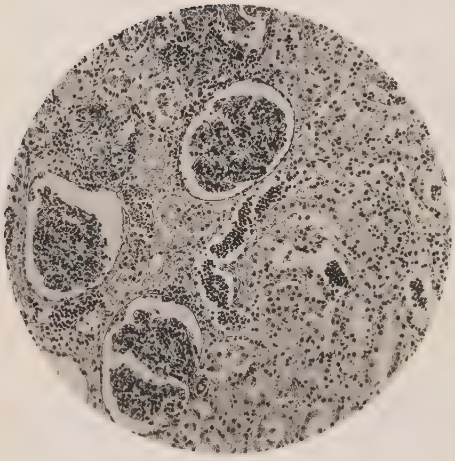


Fig. 4.

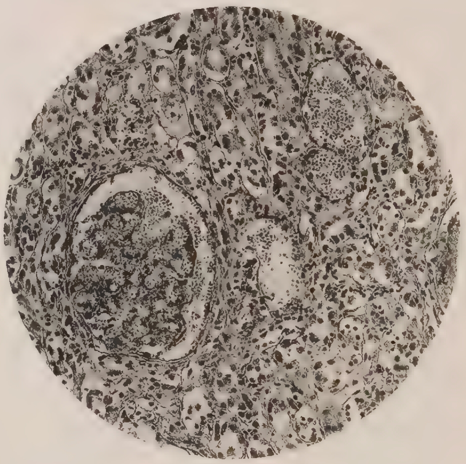


Fig. 5.

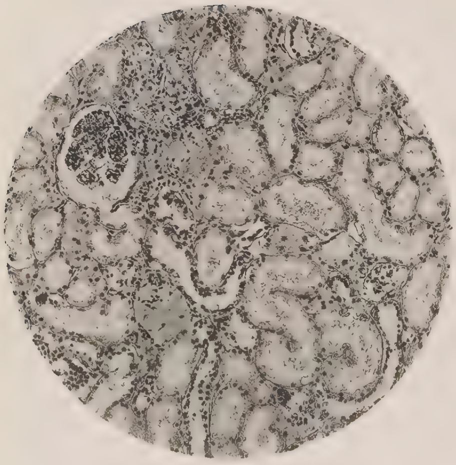
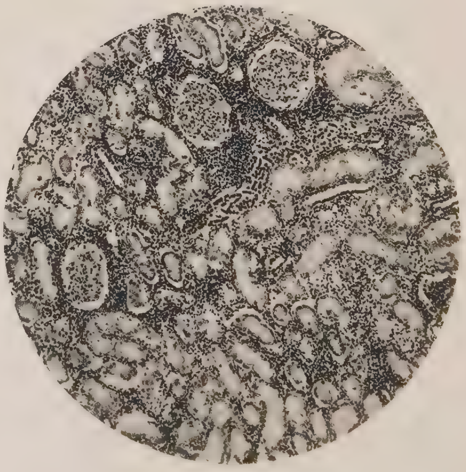


Fig. 6.



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lodged in the tubules, and *vice versa*, the greater the number of excretion foci the greater the liability of such opening into neighboring capillaries and producing embolic foci. In such cases the route of the invasion may be obscured, or its determination rendered impossible. Only in the case of each minute abscess may the mechanism of its production be ascertained, and that only so long as the embolus lies distinctly within a capillary or tubule.

As the abscess increases in size and the surrounding tissue becomes necrotic and infiltrated with pus cells, the solution of the problem increases in difficulty. The masses of organisms may occasionally be observed with the unaided eye as minute, white points or stripes at the centers of the abscesses. Practically all the purulent foci are surrounded by a red zone of hyperæmia. This zone is characterized microscopically by engorged capillaries and hemorrhage into the tissues. Further, the necrotic condition of the tissue immediately adjacent to the organism must not be overlooked. We have, therefore, three zones surrounding the masses of organisms: first, the necrotic; second, the purulent, and third, the hyperæmic. This does not, however, exhaust the numerous purulent changes produced in the kidney. As the abscesses increase in size and the destruction of tissue becomes greater, the pus cells find their way into the urinary tubules and are carried downward by the flow of urine. Thus we often find tubules in which the epithelium is intact, but whose lumena are filled with pus. (Plate VI., Fig. 3.) As in all the changes so far described the infection finds its way to the kidney through the circulation, and later is carried with the urine outward through the tubules, we speak of a descending purulent nephritis.

DESCRIPTION OF PLATE VI.

FIG. 1. Coccus embolus in a glomerulus. See p. 106. Very high, dry lens. Gram. Alum carmine. At the center of the field, the glomerulus. The loop at the periphery and above is filled with a large, branching mass. This is composed of closely packed cocci. At the edges of this mass (*magnifier*) the isolated cocci may be seen. These are minute, spherical, deeply-stained bodies.

FIG. 2. Excretion focus in the kidney. See p. 106. Middle power. Hæmatoxylin. Above a well-developed abscess. At the center a pale and elongated mass of pus corpuscles in the form of a cylinder. This occupies the lumen of a tubule. With the *magnifier* the preserved epithelium of this tubule may be made out. In the upper portion of the cylinder of pus cells a deeply stained stripe, a mass of bacteria. Surrounding the periphery of the abscess a large number of free red blood corpuscles (hemorrhage and hyperæmia).

FIG. 3. Purulent nephritis. See p. 107. Moderately high power. Hæmatoxylin. Near the center two uriniferous tubules, the lumen of which are filled with closely packed pus cells. The epithelium of these tubules is intact but partly desquamated. The adjacent tubules contain red blood corpuscles (hemorrhage). The glomeruli contain a large number of nuclei part of which are due to the presence of leucocytes (*magnifier*).

FIG. 4. Hemorrhagic nephritis. See p. 111. High power. Hæmatoxylin. To the left a glomerulus, the capsular space of which contains a large number of red blood corpuscles. The capillary loops of the glomerulus are widely distended and contain a large amount of blood. At the center and above to the right are two tubules which contain red blood corpuscles (*magnifier*).

FIG. 5. Kidney of albuminuria. See p. 110. Middle power. Hæmatoxylin. To the left and above the glomerulus with dilated capsule, which opens into a uriniferous tubule. Within the capsule of the glomerulus and in all the tubules a fine granular material (coagulated albumin). The epithelium of the tubules is preserved but distinctly flattened (*magnifier*).

FIG. 6. Leucæmia of the kidney. See p. 109. Low power. Hæmatoxylin Surrounding the glomeruli and between the tubules a large number of deeply stained nuclei. These lie in the capillaries and in the connective tissue stroma and with the *magnifying glass* are seen to be leucocytes. The epithelium of the tubules is more or less desquamated (post-mortem change).

Various classes of organisms are capable of producing these changes. In general the ordinary pus cocci (staphylococci and streptococci) are the more common offenders.

6. Pyelonephritis.

(*Ascending Purulent Nephritis.*)

As we have learned that organisms may find their way into the kidney through the circulation, so may they also gain entrance through the urinary system. This is most readily accomplished when the lower portion of the urinary tract is infected, as the bladder, and when this is associated with obstruction or stasis of the urinary excretion (paralysis or paresis of the bladder. Prostatic hypertrophy). The bacteria travel upwards through the ureter by a gradual infection of the ureteral wall and ultimately infect the pelvis of the kidney (pyelitis). Once established in the pelvis, the infection, still following the route of excretion of the urine, invades the papillæ and enters the urinary tubules (ascending purulent nephritis). Here, owing to the radiary arrangement of the straight tubes, the areas of infection become irregularly triangular in shape, presenting much the appearance of an infarct with the point coinciding with the papillus. (Plate VII., Fig. 4.) As the destructive effect of the bacteria is great, large areas of necrosis are common, and, where the infection is advanced, entire papillæ may become necrotic. The periphery of these areas is very irregular, and is surrounded by a yellowish, purulent and red hyperæmic zone, which separates the dirty, grayish necrotic centers from the surrounding as yet unaffected tissue. The majority of the organisms become lodged in the collecting and straight tubules, but may find their way upward into the cortex and there form the basis of multiple, minute abscesses. As we have seen in descending purulent nephritis that the excretion foci may be numerous and extensive, and that the organisms after passage into the kidney pelvis commonly induce pyelitis, the two processes may present identical appearances. In such cases it is impossible to determine the true nature of the process by macroscopic examination alone.

F., F. M., methylene-blue.

Low magnification shows at once the extensive necrotic areas in the medulla and papillæ, within which numerous deeply stained dots and stripes stand out prominently. (Plate VII., Fig. 3.) Higher powers resolve these masses into cylinders of bacteria, lying within the straight and collecting tubules; that is, we may reason by their length and diameter that the tubules are the only structures which could contain them. Often we may find the remains of the broken-down tubular epithelium surrounding them. Emboli of bacteria in the capillaries present a more convoluted appearance and are commonly branching. At the periphery of the necrotic areas we find the before-mentioned purulent zone, consisting of polynuclear leucocytes (Plate VII., Fig. 4), and the hyperæmic zone in which the capillaries are widely distended. We must, therefore, note the great similarity between the microscopic appearances just described and those associated with excretion foci.

On examining the miliary abscesses of the cortex, the masses of bacteria are found in the tubules in contradistinction to the abscesses of descending nephritis where the organisms lodge in the capillaries. Naturally a sharp differentiation is not always possible; for instance, where the process is advanced the

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destruction of the tissue may afford the bacteria entrance into the capillaries, or through the lymph spaces into the general circulation, wherefore a descending process is added to the already established ascending. Confronted with such a problem, the observer must have recourse to the evidence furnished by the general pathological conditions, or by purely clinical facts, and these may not always lead to a definite conclusion. In general, the distinction may be made that the descending processes are more commonly caused by cocci, while the ascending variety owe their origin in most cases to bacteria (colon bacillus and proteus). In the latter case the fact must be borne in mind that the bacteria may be very short and must not be mistaken for cocci. The bacterium coli is decolorized by Gram.

7. Leucæmia of the Kidney.

In leucæmia certain secondary changes occur in the kidney. These are similar to those already described under the same heading in the liver. The kidney is enlarged, the cortex sprinkled with grayish dots similar in appearance to tubercles. The characteristic tracery of the cortex is obliterated.

F., F. M., N. S., or F. A., or sublimate. Embed.

By low power the tissue shows dark spots of varying size scattered through the section. The large veins are filled with dark masses and the capillaries between the tubules are accentuated by their dark color. (Plate VI., Fig. 6.) These variations from the normal (high power) are due to changes in the composition of the blood, which is found to contain an increased number of leucocytes, the character and per cent. of which varies in each individual case. (See Blood.) In severe cases the predominance of white cells may be so great that the capillaries appear as if injected with leucocytes. As the arteries are usually empty the alteration of the blood is best observed in the venous system. The irregular, deeply-stained areas are now resolved into accumulations of leucocytes in the connective tissue structure surrounding the vessels. The macroscopically visible nodes are likewise found to consist of accumulations of leucocytes in the tissue. How many of these cells have migrated from the vessels, or to what extent they have increased in number within the tissue, may not be determined. The leucæmic kidney must be distinguished from a possible advanced purulent or interstitial nephritis. This is easily accomplished by examining the blood within the organ, which in the last-named conditions is unchanged. Likewise the character of the cells in the tissue must be observed.

In leucæmia the foreign cells scattered through the tissue are usually of the mononuclear variety. These are somewhat larger than the round cells which appear in the tissue in interstitial nephritis. In purulent nephritis the leucocytes are of the polymorphonuclear variety.

8. Acute Nephritis.

The characteristic clinical symptom of this change is the presence of albumin in the urine. The simplest metabolic disturbance (physical exhaustion) and all intervening grades, to the most serious general disease, have the ability of so injuring the epithelium covering the glomeruli, as to permit the transudation of the soluble albumin of the blood into the urine, and this without producing any appreciable change either macro- or microscopic. The detection of the albumin in the tissue may be accomplished by the application of one of the agents employed for the same reaction in the urine, namely, heat. Small pieces of tissue

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are thrown into boiling water for a few seconds, hardened in increasing alcohols, embedded and cut. Simple stains.

Low power. The first glance shows the capsular spaces of the glomeruli and the urinary tubules to be filled with a foreign material. Higher magnification shows this material to consist of a fine granular and stringy mass, which is no other than coagulated albumin. (Plate VI., Fig. 5.) The dilatation of the glomeruli capsules and urinary tubules indicates an excessive excretion of urine. In spite of the amount of albumin in the urine, we are unable to determine any alteration in the epithelium of the glomeruli, so that we are only permitted to suppose that the cells have been subject to some chemical alteration, or that if structural, the changes are too subtle for detection with our present means of observation. The epithelium of the secreting portions of the uriniferous tubules have a perfectly normal appearance. As the excreted albumin passes down through the urinary tubule, it not uncommonly happens that the fluid constituents are removed by absorption and that the albumin is deposited in the tubes in the form of so-called hyaline cylinders. This cylinder formation is found as high up as the Loops of Henle. In most cases of acute nephritis there are more or less distinct macroscopic changes. The organ is enlarged, contains more fluid, and the tissue is more brittle than normal. The swelling of the tissue is especially marked in the cortex, which on the cut surface rises above the medulla. It presents on close examination all of the various changes associated with cloudy swelling and fatty degeneration. The cortex is extremely pale and anæmic as compared to the hyperæmic medulla.

The microscopic examination of *fresh tissue* shows the various changes characteristic of parenchymatous or fatty degeneration. Cloudy swelling, vacuolous degeneration, granular disintegration, fatty degeneration and necrosis of the epithelium, especially in the convoluted portions of the tubules, are conditions most commonly encountered. Even changes of the epithelium covering the glomeruli may be present.

Harden in formaline, F. M., N. S. or van Gieson. Or formaline alcohol, F. M. or embed, fibrin stain. Or harden in boiling water or Flemming, Hermann or sublimate, etc. Various nuclear or protoplasm stains.

In hardened preparations the spaces in the stroma of the kidney are found to be filled either with fibrin or albumin. Occasionally accumulations of leucocytes in the stroma may be seen in the neighborhood of the glomeruli. Not infrequently the process is of a hemorrhagic character (hemorrhagic nephritis). The hemorrhagic form frequently develops in the course of severe general disease and is usually the result of direct injury to the vessels in the organ.

On the cut surface after removal of the capsule, the tissue is studded with minute black and deep red spots which stand out plainly from the intervening tissue, which, owing to accompanying fatty degeneration, is often of a yellowish tinge. Cut surfaces of the substance present the most variegated appearance, the red and yellow of the tissue standing out in distinct contrast (large variegated kidney).

Formaline, F. M., N. S., or van Gieson or sublimate, embed, hæmatoxylin eosin, or formaline alcohol, F. M., or embed, fibrin stain.

Examination of the section with low power discloses the fact that the areas of hemorrhage are for the most part composed of dilated channels filled with blood. These canals are lined with epithelium and are uriniferous tubules, the epithelium of which is more or less flattened, probably from the pressure of the extravasated blood in the lumen. (Plate VI., Fig. 4.) In the examination of these tubules, great care must be taken not to confuse them with the dilated capillaries by which they are surrounded. Careful examination will

PLATE VII.

Fig. 1.

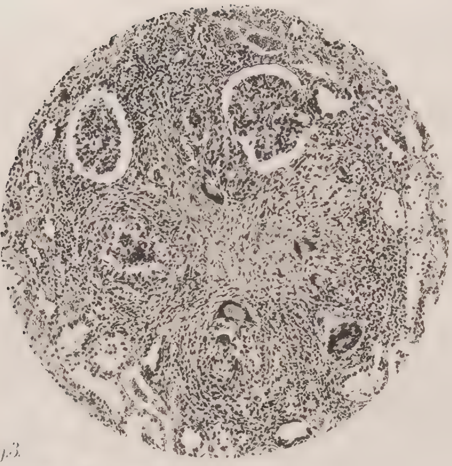


Fig. 2.

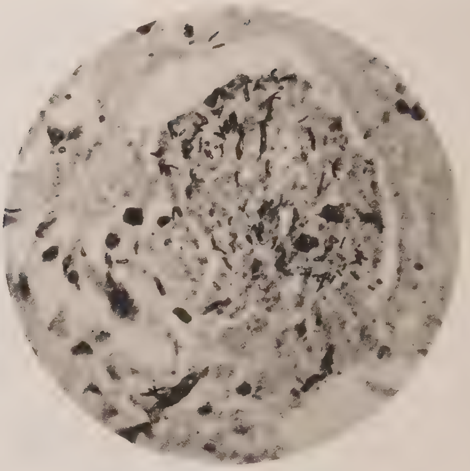


Fig. 3.

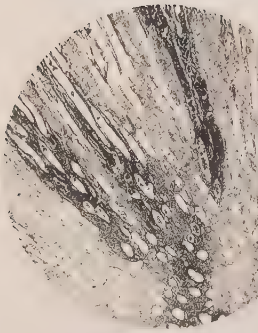
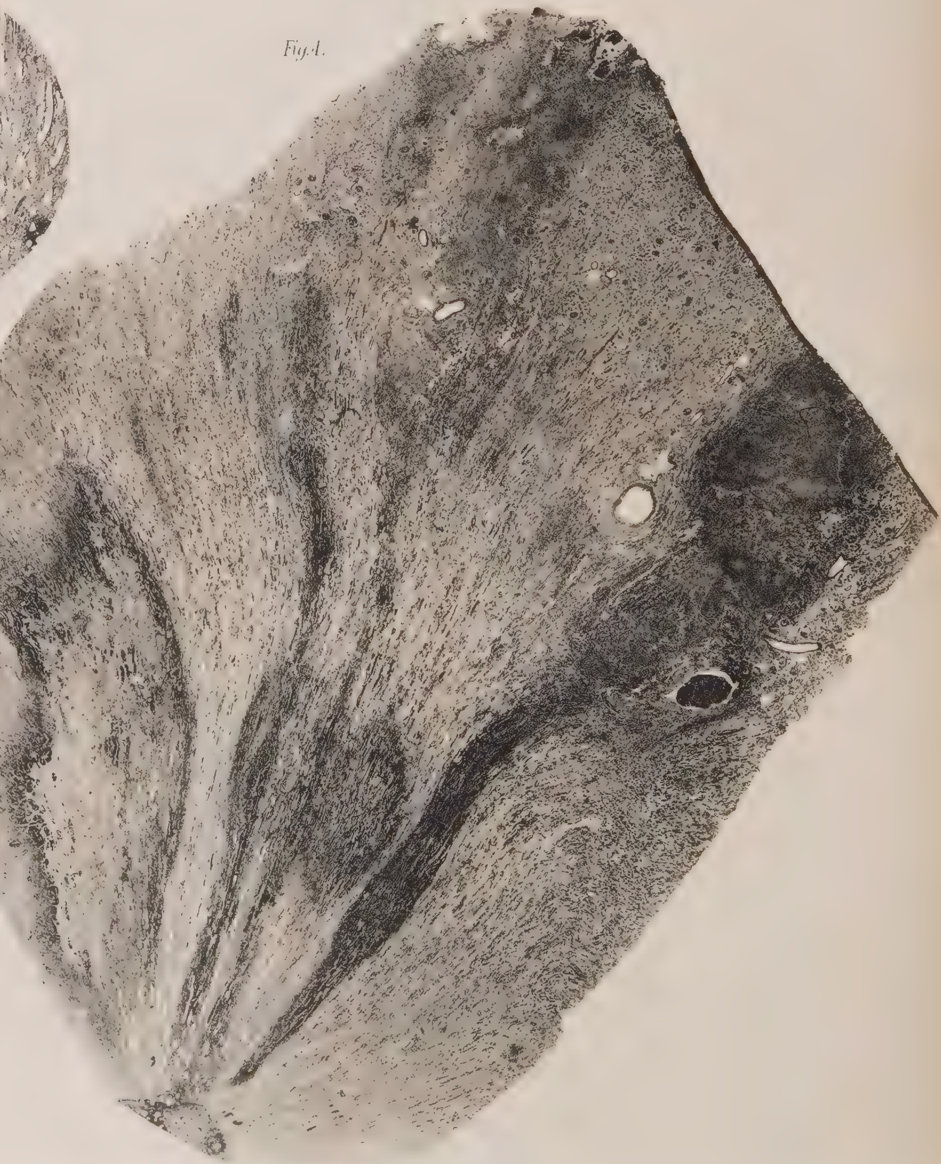


Fig. 4.



show that the layer of protoplasm, even in the flattened epithelium of the tubules, is thicker than the epithelium of the capillaries and that the nuclei are placed closely together. How are we to account for the presence of the hemorrhage in the uriniferous tubules? The extensive engorgement of the adjacent capillaries might lead to the conclusion that the blood had been extravasated from them and had thus found its way into the urinary canals. Such a possibility may not be excluded but a careful examination of the tissue will show another and a more fruitful source in the glomeruli. If the preparation be carefully searched no difficulty will be found in locating glomeruli in which the capillaries are widely distended and in these the space within the capsule of Bowman will be found to contain free blood corpuscles. (Plate VI., Fig. 4.) Naturally in such cases the blood is carried with the urine through the entire urinary system, and the source of the hemorrhage may not lie in the section examined. During the passage of the blood through the canals it undergoes changes, in particular the hæmoglobin is thrown into solution and taken up by the urinary epithelium as granules of yellow pigment. Occasionally the epithelium of a tubule may be the seat of such pigmentation while the lumen is filled with well-preserved blood globules. Pigment is likewise commonly deposited in the interstitial connective tissue. Beside the changes which lead to hemorrhage, the glomeruli in all cases of advanced nephritis are subject to other alterations, which we may profitably consider at this point.

The Malpighian corpuscle consists of the glomerulus and the surrounding capsule lined with epithelium. Inflammatory alterations of either the glomerulus or the capsule may be present. Cloudy swelling, fatty degeneration, necrosis and desquamation of the epithelium of the capsule are frequent. The desquamated epithelium mixes with the albuminous exudate within the glomerulus capsule, which likewise contains leucocytes and isolated red blood corpuscles. These are the common degenerative changes which affect the capsule. The glomerulus proper, when carefully examined, is likewise subject to various degenerative changes, the most common of which is hyaline degeneration, which may only affect single loops of the glomerulus, and which gives the capillary a thickened and homogeneous appearance. The degeneration may affect either the wall of the capillary or may consist of deposits of hyaline material within the lumen, which may be of varied composition. Not uncommonly they consist of leucocytes, blood plates or fibrin masses, which have become closely packed

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DESCRIPTION OF PLATE VII.

Fig. 1. Tubercle of the kidney. See p. 119. Middle power. Hæmatoxylin. Above two glomeruli. At the periphery well-preserved uriniferous tubules. At the center a reticulated tubercle surrounded by epitheloidal cells and containing several giant cells (*magnifier*).

Fig. 2. Glomerulus from a kidney affected by miliary tuberculosis. See p. 119. Oil immersion. Recent lodgment of tubercle bacilli in the loops of a glomerulus. These appear in the glomerulus as slender rods, many of them crossed (*magnifier*). Above in the capsular space two bacilli. The structure of the glomerulus and the surrounding tissue necrotic. Here and there an occasional nucleus.

Fig. 3. Pyelo-nephritis. See p. 108. Low power. Hæmatoxylin. Section through the apex of a papillus. Below and to the right cross section with collecting tubules. To the right and above two collecting tubules filled with deeply stained masses of leucocytes and bacteria.

Fig. 4. Pyelo-nephritis. See p. 108. Very low power. (Micro-collinear.) Section of a papillus and adjoining cortex. Below and extending up into the cortex are tracts of necrotic tissue. To the left a large area of necrosis surrounded by a zone of purulent infiltration. The extension of the process through the straight tubules may be seen in the dark bands to the right. Above the glomeruli of the labyrinth (*magnifier*).

and undergone hyaline transformation. In hemorrhagic nephritis when the tissue is stained with Weigert's fibrin method, a fine fibrinous network may be seen within the loops of the glomerulus. The form of hyaline change which affects the wall of the capillary not uncommonly gives a fibrin reaction (fibrinoid degeneration). A certain number of cases of acute nephritis (scarlet fever, diphtheria, and other infectious diseases), do not present the typical features of parenchymatous degeneration. The organ is enlarged, the contents more fluid than normal but the yellowish coloration and yellow dots and cloudy appearance of the tissue are missing. The cortex of the organ has a dull gray appearance with here and there whitish dots. The line of demarkation between the medulla and cortex is more or less obscured.

Formaline, F. M. Horizontal and longitudinal sections, or formaline alcohol, F. M. or embed, plasma cell stain.

When examined under low power the section shows certain dark areas usually localized near or under the capsule and about the glomeruli, and at the bases of the pyramids and around the smaller veins. Under high power these dark areas are found to be due to accumulations of cells in the interstitial tissue between the tubules (acute interstitial or fresh granulating nephritis). These cells are found to closely resemble lymphocytes. Some, however, are slightly larger. When stained with Unna's method, the larger cells are found to be plasma cells. To what extent this accumulation of cells is due to transmigration of lymphocytes from the small vessels or to what extent they are derived from the fixed elements of the tissue cannot be determined. Degenerative changes of the epithelium are slight or entirely absent. The glomeruli may be either intact or show various inflammatory changes.

9. Subacute and Chronic Nephritis.

A sharp boundary between acute and chronic nephritis cannot be drawn. The characteristics of chronic nephritis consist in degeneration and disappearance of the epithelium of the organ and certain productive processes affecting the connective tissue of the blood vessels, as well as special degenerative changes which affect the glomeruli. The degeneration and destruction of the epithelium usually lead to shrinkage and decrease in size of the organ.

All chronic inflammatory change produces ultimate destruction of the special structure affected, which leads to decrease in size of the organ, when the loss of tissue is not replaced by some foreign substance (*i. e.*, amyloid). The intensity of the process is not the same at all points within the kidney structure. This is due to unequal distribution of the irritant and variation in the changes which it produces, causing at one point excretion of abnormal substances into the tissue and at others destruction of the tissue. As a result, the contraction is very irregular, in many cases the surface of the organ presenting a granular appearance. We may, therefore, expect, in proportion to the duration of the process and the character of the special tissue affected, great variety in the appearances which are associated with chronic nephritis. If, at the beginning of the process the epithelium is extensively affected by degenerative processes, we speak of

1. **Chronic Parenchymatous Nephritis.**—This condition is usually a continuation of acute parenchymatous nephritis. Although the process is chronic there is no tendency to contraction. In general, the organ presents macroscopic appearances similar to those found in acute nephritis, except that the swelling

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of the organ is more marked and the fatty degenerative changes predominate over those of cloudy swelling. The yellowish color of the tissue and the mottling of the cortex is more accentuated, for which reason kidneys thus affected are described as large, white or yellow kidney. Not uncommonly the change is associated with minute hemorrhages in the cortex, which gives the tissue a brilliant variegated appearance (so-called large red kidney). In advanced stages grayish white areas may be found in the cortex, which obscure the characteristic structural markings. These are due to interstitial changes. In advanced cases the destruction of the parenchyma leads to numerous depressions and contractions of the surface (secondary contracted kidney).

Microscopic examination may be made either with *fresh or hardened material* and on examination the changes in the tissue are found to closely resemble those already observed in acute parenchymatous nephritis, the principal distinction consisting in more or less widespread proliferative changes in the connective tissue. When the section has been made horizontal to the surface of the organ, the examination, even with low power, discloses dark areas which lie between the less deeply stained sections of the tubules. Under high power these dark areas are resolved into accumulations of lymphocytes and round cells, and are found to be localized about the smaller vessels, between the tubules or thickly massed about the capsules of the glomeruli. These appearances are, therefore, with the exception of the extensive degenerative changes in the epithelium, very similar to those found in acute interstitial nephritis. The chronic character of the process is shown in the areas of contracted tissue which, in the later stages of the process, become more widespread and present all the appearances found in true contracted kidney, which shall be described later.

In subacute forms of nephritis attempts at regeneration may be found in the epithelium of the tubules. These attempts are evidenced by the presence of karyokinetic figures in the epithelium and occur usually in the neighborhood of defects. Not uncommonly large masses of protoplasm containing multiple nuclei may be found. They present a syncytial appearance. Beside proliferative changes in the connective tissue and stroma of the organ, more or less widespread changes of the glomeruli may be found in these cases of chronic nephritis. When these changes in the Malpighian bodies predominate, or, are the sole change in the tissue, we speak of

2. Chronic Glomerular Nephritis.—If the cut surface of a normal kidney is held between the observer and the light at such an angle that the light is reflected from the kidney surface, it is possible to demonstrate minute grains in the kidney substance, which are the glomeruli. This is a matter of some delicacy and requires acute observation. In some cases the glomeruli may be seen without difficulty as distinct bodies, and in other cases it is absolutely impossible to observe them with the unaided vision. The parenchyma of the organ in these cases may show no alteration whatever (as in scarlet fever). The microscopic examination may disclose the most widespread changes in the glomeruli.

Formaline, F. M., N. S., or elastica stain or van Gieson, or Zenker, embed in celloidin, Mallory's connective tissue stain.

The changes in the glomeruli in chronic glomerular nephritis are practically an advanced stage of those found in acute nephritis (degeneration of the capsule epithelium, hyaline changes in the capillaries).

The cases which more commonly escape investigation are those in which the kidney macroscopically appears unchanged, as in scarlet fever, but where the microscopic examination reveals serious alterations of the glomeruli. (*M. F., F. M., N. S.*) If we investigate first that form in which the epithelium of the capsule is principally affected, as in scarlet fever, we find (low power) the space within the capsule dilated and filled with darkly stained masses, which (higher power) are resolved into accumulations of cells (Plate VIII., Fig. 1). These are none other than newly proliferated and desquamated epithelial cells from the capsule. While in the normal glomerulus the capsule of Bowman is covered by a barely demonstrable layer of flattened epithelium, the epithelium is here swollen and plainly visible. As the desquamation progresses, the epithelium fills out and dilates the space within the capsule, and finally pushes its way into the opening of the uriniferous tubule. In advanced cases the cells form a homogeneous mass which is sufficient to wall up the glomerulus, destroying its function and causing it to undergo degeneration. In isolated cases the proliferation of the epithelium is not so great, but is associated with proliferative changes in the connective tissue surrounding the capsule and accompanying the vessels of the glomerulus. In such cases the opposing surfaces may adhere (capsulitis adhesiva) at different points, forming divisions in the capsular space which, through proliferation, are lined with epithelium. The space within the capsule may be thus divided (Plate VIII., Fig. 2), much after the manner of the sacculations which occur in pleuritis.

The most frequent affection of the glomerulus is the hyaline transformation. (Plate VIII., Fig. 4.) The hyaline change affects primarily the walls of the capillaries, which are transformed into thickened tubes which gradually close, leaving the glomerulus a mass of homogeneous substance, with here and there an isolated connective tissue cell to mark the divisions between the vessels. The circulation in the vessels and the secretion of the glomerulus is thus destroyed. The epithelium of the capsule perishes, the opposing surfaces of the capsule unite, and with the connective tissue surrounding them adhere to the hyaline mass which represents the glomerulus. The hyaline glomerulus thus appears as if embedded in a ring of connective tissue. Later the glomeruli frequently become impregnated with lime salts, a condition which may be detected macroscopically, the glomeruli appearing as distinct, minute, white bodies, best observed on the surface of the kidney.

It must, however, be observed that a great many of the white dots are not calcified glomeruli but minute cysts containing hyaline material which has undergone calcification. These cysts are for the most part composed of glomerulus capsules which have been filled with hyaline masses. Not uncommonly the tuft of blood vessels of the glomerulus may be detected in the walls of these cysts, although much deformed. The remainder of these calcified cysts are produced by occlusion and dilatation of segments of the uriniferous tubules.

An important but very rare form of disease of the glomeruli is found in subacute cases of nephritis, where the glomeruli may be seen macroscopically as large granular bodies plainly demonstrable on the cut surface of the organ. They may be examined in fresh sections or by the aid of teased material. The glomeruli are greatly increased in size and present a plumper appearance than normal, and single capillary loops contain a larger number of nuclei than normal. The cause of this increase of the nuclei in the capillary loops is difficult to determine, even with the aid of hardened preparations. (Sublimate or Flemming. Paraffine sections.) The enlargement of the single loop is usually due to occlusion of the lumen by masses of leucocytes or cells derived from the capillary endothelium by proliferation.

3. Chronic Interstitial Nephritis.—The result of this process is the so-called true or common contracted kidney (granular atrophy of the kidney). The kid-

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ney, which is much smaller than normal, presents a most characteristic alteration of the surface. This consists of a fine granulation, produced by minute elevations of yellowish or grayish white aspect, alternating with deep red depressions of the surface. Section of the organ reveals a distinct alteration of the usual division into cortex and medulla, the boundary being partly obliterated and showing marked irregularities suggestive of a mixing of the two structures. The glomeruli are not so easily demonstrated as in the normal organ, being often invisible to the closest scrutiny. The addition of areas of brownish and yellow color disseminated irregularly through the tissue gives the whole a most unusual appearance. Here and there larger and smaller cysts may be detected in the tissue. Withal the cortex may be greatly reduced in thickness, measuring but 2–3 mm.

Formaline, F. M., N. S. or elastic fiber stain, or van Gieson. Zenker. Embed in celloidin. Mallory's connective tissue stain.

The lower the magnification and the greater the area of tissue brought within the field, the more characteristic the appearance. First, the surface of the organ presents conditions of interest. We see plainly the irregularities of contour representing the elevations and the depressions of the surface. The elevations are particularly easy to distinguish from the depressions by their structural difference, a differentiation between them being easily traced for some distance beneath the surface and well into the kidney structure. The elevations attract our attention because they are made up of widely dilated uriniferous tubules, while the depressions consist of more compact tissue, within which the characteristic kidney structure is more or less obliterated. (Plate VIII., Fig. 3.) In the segments of tissue beneath the elevations the glomeruli are plainly seen, while in the intermediate areas they are not apparent, and may be first recognized as markedly altered and shrunken bodies. Higher magnification enables us to obtain a clearer idea of the details in these widely different areas of kidney structure. First, the areas associated with the peripheral depressions. Here the tissue structure may be practically hidden by an advanced round-cell infiltration which obliterates all boundaries and invades all portions of the tissue. Careful examination of these areas of marked round-celled infiltration discloses the presence of minute tubules lined with flattened epithelium. These are the remains of the uriniferous tubules which have become markedly atrophic owing to the pressure to which they have been exposed by the proliferating interstitial tissue.

Occasionally the tubules are so reduced in width and the epithelium so altered that we may remain in doubt as to whether or not the remains of a tubule are under examination. The glomeruli are likewise shrunken and show advanced hyaline degeneration, proving that the function of the structure has long been obliterated. Great difficulty surrounds the determination of the question of which constituent of the renal structure was primarily affected, whether the glomeruli were first affected and the secretion ceasing, the tubules became secondarily atrophic, or whether the process, beginning in the connective tissue, produced through pressure the destruction of the parenchyma, impeding and occluding the flow of urine, thus reacting upon and rendering atrophic the glomeruli, or whether all portions of the reniculus were alike primarily affected by some irritant.

In contrast to the areas in which the function of the parenchyma has been lost, the portions beneath the elevations show marked functional activity. The evidence of a form of compensatory hypertrophy is found not only in the enlarged glomeruli but in the epithelium of the tubules, which greatly exceeds in size that of the normal parenchyma. To be sure, the yellowish color of these areas indicates that the hypertrophic structure has suffered serious alteration which finds expression in marked fatty changes of the cells.

That the kidney structure has suffered an actual contraction in the sunken areas is proven by the close proximity of the hyaline glomeruli. We have, therefore, a primary focal alteration of the kidney structure indicated by the depressed surface with dependent segments. The intervening areas show an advanced form of compensatory hypertrophy. The deep red color of the contracted segments is easily explained by the relatively greater vascularity of these areas. It is easily understood that the widespread injury of the glomeruli would induce marked disturbance in the circulation of the kidney, especially in the tension of the arterioles and arteries. Such disturbance where long continued leads to alterations of the walls of the arteries known as arterio-sclerosis, which expresses itself for the most part by marked thickening of the intima. The greater the thickening of the intima the smaller becomes the lumena of the vessels, with proportionate decrease in the blood supply.

The slower the alteration of the blood supply the greater the development of cicatricial tissue and the more marked the contraction and resulting atrophy.

Occasionally, where the process advances very slowly, extensive segments of tubules may become surrounded while the epithelium is still capable of secretion, and large cystic dilatations result.

The contents of these cysts are variable, being often aqueous, or taking a homogeneous stain, or occasionally presenting a concentric striation. (Colloid secretion or metamorphic products of the epithelium.)

It is interesting to observe the status of the elastic tissue in the organ. In all the contracted areas in the kidney a great increase in the amount of elastic tissue is apparent. This is most marked about the glomeruli where numerous elastic fibers surround the capsule of Bowman. Where the atrophy of the tissue is excessive the proportion of elastic tissue may be very great. In cases of advanced hydronephrosis, the portion of the hydronephrotic sac which is made up of the kidney structure, may be composed for the greater part of elastica. In such cases where the atrophy of the organ is very advanced only an occasional remaining glomerulus or deformed uriniferous tubule may be left to indicate the location of the atrophic kidney structure.

10. Arterio-Sclerotic Contracted Kidney.

We have seen that in granular atrophy of the kidney the vessels of the contracted areas showed advanced arterio-sclerosis, resulting from primary injury of the glomeruli. The sclerotic change in the vessels may likewise be primary, in which case it is usually part of a general change affecting the entire vascular

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system. Naturally diminution of the lumen of a vessel, or obliteration of the lumen, leads to widespread nutritional changes in the tissue which it supplies. The course of the alteration of the blood current is, however, too gradual to produce the changes already studied in the so-called pale infarct of the kidney. As in infarcts the necrosis of the tissue is pyramidal in form, so here the altered segment follows the distribution of the blood supply of the artery, and consists of a conic segment with the base placed at the surface. The contraction of these areas produces irregular depressions of the surface dependent in extent and form upon the size of the vessel and the degree of alteration of the lumen.

Occasionally a single crater-like depression is formed. Again an entire half of the organ is atrophic, or the entire organ may be irregularly contracted and the surface of the contracted areas may show granulation, as in granular atrophy. On section the marked atrophy of the cortex in the depressed areas and the conical form of the affected areas becomes apparent. The contracted portions are more reddish in appearance than the uncontracted portions, which produce relative elevations where the contractions are multiple. The arteries of the organ are of great size and because of the rigidity of their walls, stand out prominently on the cut surface of the organ. In the contracted areas the vessels are placed relatively near the surface. In short, the condition is one analogous to granular atrophy, except that larger vessels and larger areas are primarily affected. The unequal distribution of the arterial changes produces the coarse depressions and the relative elevations of the surface.

F., F. M., N. S., or van Gieson, or Elastic Fiber Stain.

We examine first with low power the line of junction between medulla and cortex, for here lie the larger arteries. These are easily found and the alterations of the vessel walls, especially the thickening of the intima, may be readily studied. (Plate IX., Fig. 2.) The student must avoid the error of mistaking oblique sections of the arteries in which the intima occupies undue prominence, for arteries affected with actual thickening of the intima. The oval character of the lumen and the distinct thinning of the intima toward the lumen serve to easily distinguish an oblique section of an artery even when the intima is affected, as in Fig. 2, Plate IX.

Toward the surface of the organ the vessels become smaller, but the thickening of the intima more pronounced; in some vessels the lumena are completely closed (endarteritis obliterans). The natural sequel to contraction and occlusion of the vessels is marked alteration of the glomeruli which they supply. (Plate IX., Fig. 2.) In the contracted areas all, or nearly all, of the glomeruli are the victims of hyaline degeneration. Then follows atrophy of the tubular epithelium, round-cell infiltration, connective tissue growth and contraction, in short, all the processes found in granular atrophy. The gradual transformation from the periphery of the altered areas to the unaltered structure (which may likewise show compensatory hypertrophy) of the kidney present no differences from those already described. Of interest are the large number of hyaline cylinders found in the straight tubules of the medulla.

One could easily suppose these masses to be the altered secretion of the epithelium of the tubules, which through the failure of the glomerular secretion have not been washed away and have taken on a hyaline or colloid condition.

11. Hydronephrotic Contracted Kidney.

The forms of contracted kidney thus far described have been those in which the cortex has been mainly affected. There is another form of contracted kidney produced by pressure exerted upon the kidney structure from obstruction of the urinary flow. When the accumulation of fluid occurs in the kidney pelvis the pressure thus exerted causes a characteristic flattening of the papillæ and disappearance of the pyramids. At this stage of the process the cortex may be unaffected, the medulla nearly obliterated. As the dilatation progresses the stretching of the cortex and pressure exerted upon it produces atrophy, followed by destruction of the parenchyma. The histological findings vary with the degree to which the process has advanced. The following may be considered characteristic of cases at a moderately advanced stage.

F., F. M., N. S., or van Gieson or elastic fiber stain.

Low power shows at once a distinctive difference between the atrophic cortex and the changes detailed under the various forms of contracted kidney.

The glomeruli are intact, the nuclei distinct. The space within the capsule of Bowman is widely dilated, the convoluted tubules likewise, due to the increased pressure resulting from the obstruction to the urinary current. The glomeruli lie very close together, a proof of distinct reduction of the intervening tissue. In many places we find a great decrease in the number of uriniferous tubules, and in places (best confirmed by high powers) an entire absence of tubules. Such areas show a marked round-cell infiltration, representing, no doubt, an attempt of the connective tissue to replace the obliterated structures. The epithelium of the remaining tubules presents marked evidence of degen-

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DESCRIPTION OF PLATE VIII.

FIG. 1. Glomerular nephritis. See p. 114. Middle power. Hæmatoxylin. At the periphery to the right a nearly normal glomerulus, the capsule of which may be distinctly made out with the *magnifier*. Above and at the periphery a glomerulus, the capsular space of which to the right is filled with desquamated epithelium, whereas to the left the capsular space is covered by a simple layer of epithelium. To the left and below, a glomerulus surrounded by a concentric mass of epithelial layers. The uriniferous tubules above at the center and below, contain hyaline cylinders in cross section.

FIG. 2. Glomerular nephritis. See p. 114. Moderately high power. Hæmatoxylin. Above and below a glomerulus each of which is adherent to its capsule. In the lower glomerulus the upper left hand portion of the capsular space is isolated by a band of connective tissue and lined with capsular epithelium (*magnifier*).

FIG. 3. Granular atrophy of the kidney. See p. 115. Very low power. Hæmatoxylin. Above the capsular surface of the kidney. At the center of the capsular surface a slight, depressed, atrophic portion, beneath which the parenchyma of the kidney is markedly contracted. The tubules may be made out with a *magnifier*, as fine, markedly compressed structures. In this area the glomeruli are scarcely visible. To the right and left of the contracted area the tubules show marked dilatation, which on the surface produces two elevations, one to the right and one to the left. The glomeruli in these areas are definite and somewhat enlarged.

FIG. 4. Hyaline degeneration of a glomerulus. See p. 114. High power. Hæmatoxylin. Within the capsular space and below, the remaining structures of the capillary loops may be made out. Above the capsular space is filled with a hyaline mass in which are embedded a few slender nuclei. This is adherent to the capsule of Bowman, which is well defined.

FIG. 5. Amyloid contracted kidney. See p. 120. Middle power. Hæmatoxylin. The glomeruli are enlarged and pale. A large number of glomeruli in so small a field indicate a marked contraction of the organ. The connective tissue stroma shows a well-developed round-celled infiltration. At the center a dilated vessel with thickened intima (amyloid vessel).

FIG. 6. Amyloid degeneration of a glomerulus. See p. 121. High power. Hæmatoxylin. The endothelium of the capillary loops may be well seen within the structure of the glomerulus. With the *magnifier* the walls of the capillaries may be made out. Between these the homogeneous amyloid material. The capsular space is more or less obliterated. To the right and below a well-developed round-celled infiltration between the uriniferous tubules.

PLATE VIII.

Fig. 1.

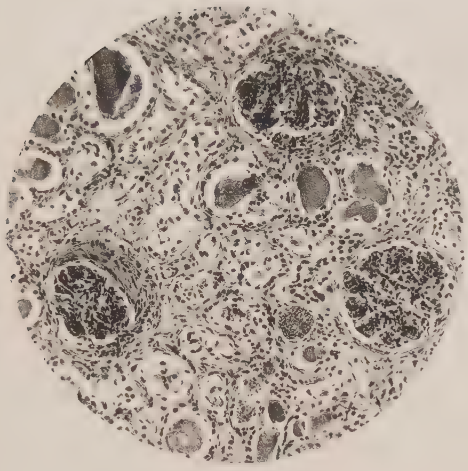


Fig. 2.

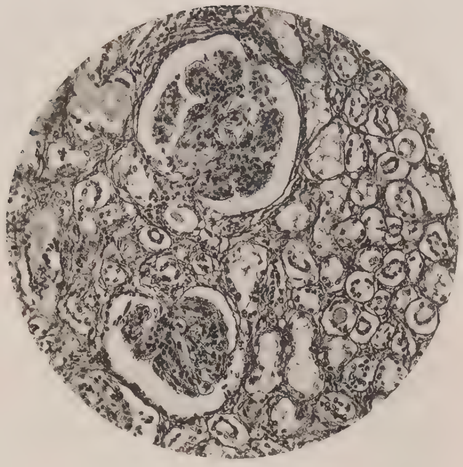


Fig. 3.

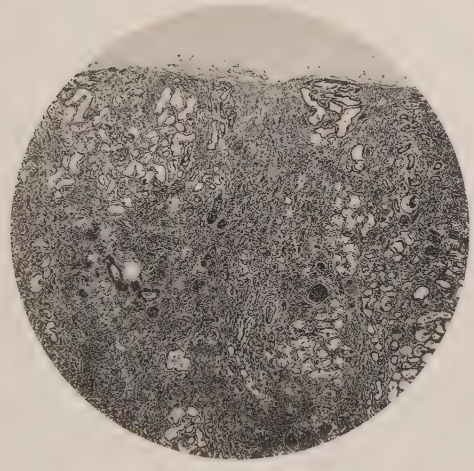


Fig. 4.

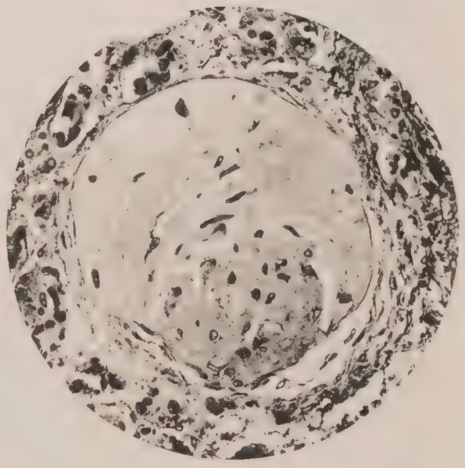


Fig. 5.

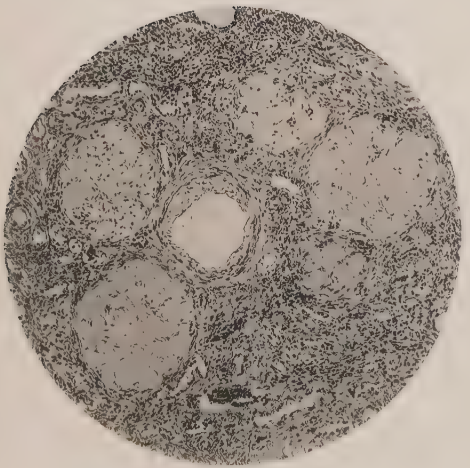
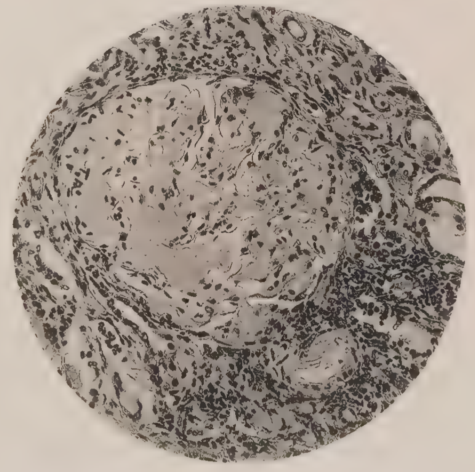


Fig. 6.



eration and atrophy extending through their entire course. The secondary alterations of the tissue in hydronephrosis are not always as characteristic as the primary. In some cases the glomeruli may become atrophic and degenerated early in the progress of the disease, the vessels, due to increased intervascular pressure, may develop sclerotic changes, and the round-cell infiltration of the connective tissue structure may be excessive. In such cases the similarity of the findings to those described under arterio-sclerotic contracted kidney may render an easy determination of the nature of the process impossible.

12. Tuberculosis of the Kidney

Occurs most commonly as part of a general miliary tuberculosis. The entire kidney particularly in the cortex is filled with minute tubercles. On the surface of the kidney these may be detected as minute, grayish bodies, a certain number of which have yellowish centers. Occasional minute, infarcts of the tissue may be found which are due to emboli in the vessels, or to the development of intima tubercles causing occlusion of the smaller vessels. M. F., F.M., carbol fuchsin, methylene-blue.

Inspection of the section with low power reveals the tubercles as small, deeply stained areas within which occasional poorly stained spots indicate caseation. Here and there within the tubercle, minute, red dots are observed which, under the oil immersion, or dry lens of sufficient defining power, prove to be masses of tubercle bacilli. The finer structure of the tubercles is the same as those developing in other glandular organs. According to the acuteness or chronicity of the infection, the number of bacilli present, and the virulence of the organisms, the predominating form may vary from the small accumulations of epitheloidal cells with marked peripheral accumulations of round cells to larger reticulated structures with varying numbers of giant cells. (Plate VII., Fig. 1.) Not uncommonly the tubercles develop in the centers of the glomeruli, in which case the capsule of Bowman may be filled with tuberculous material and giant cells. Caseation, which may be present in the smallest tubercle, indicating the destructive action of the bacilli, may likewise make its appearance late in the process, and where widespread, may, through a process of softening, produce cavities within the tubercular areas.

It would divert us too widely to describe in detail the various acute, subacute, and chronic forms of tuberculosis of the kidney. The mechanism of tuberculous infection of the kidney is of interest. Evidence of the avenue which the bacilli follow may be found by examining the tubercles. Especially in acute infections, necrotic remains of glomeruli will be found at the centers of the tubercles, and within them masses of bacilli. (Plate VII., Fig. 2.) When the structure of the glomerulus is sufficiently preserved, these will be seen to occupy the lumena of the glomerular vessels. Unquestionably many of the organisms have passed through the walls of the glomeruli into the uriniferous tubules. Those remaining within the glomeruli have produced necrosis and the development of that tissue complex designated a tubercle. Those which have found their way through the glomeruli may become lodged at any point within the uriniferous tubules, producing areas of necrosis and tubercular structures analogous to the excretion foci of purulent nephritis. These may be recognized by the location of the masses of bacilli within the tubules, which may in turn be identified by the remains of tubular epithelium. Prolonged search seldom fails to locate emboli of bacilli within the capillaries of the glomeruli, or cylinders of organisms within the tubules. Here we are dealing with early stages of the infection. Where the process is advanced the necrosis becomes general, and the bacilli proliferate unrestrained through the entire organ. A further form of tuberculous infection of the kidney is one analogous to ascending purulent nephritis. In this case the process is an ascending infection in which the bacilli enter the kidney structure by way of the papillæ, producing necrosis and caseation, ascending through the straight tubules of the medulla and spreading out in the cortex in the form of diverging rows of tuberculous foci. These may later coalesce and lead to the formation of large areas of tuberculous caseation. In spite of the chronic character of tubercular pyelonephritis, the areas of necrosis and conglomerate tubercles contain large numbers of bacilli.

13. Kidney of Syphilis. Amyloid Degeneration.

Where one encounters a kidney the surface of which presents a single deep depression, a suspicion of syphilis may be justly awakened. Naturally, the observer must exclude depressions of the surface caused by old infarcts, or isolated depressions produced by sclerosis of a single vessel of the kidney. Both of these conditions may closely resemble the destructions of tissue produced by syphilitic change, and it is only when the tissue contains well-defined gummata within the degenerated tissue or where a clear history of syphilis has been obtained that the differentiation may be made. The probability of the syphilitic character of the process is greatly increased when the contracted organ is affected by amyloid change, and other probable cause of the degeneration (as tuberculosis, grave disease of bone, etc.) is wanting.

The nature of amyloid degeneration has been indicated in the paragraph devoted to that change in the liver. The amyloid kidney (with or without diminution of size) is characterized by increase in weight and solidity, the ease with which it may be cut, and the transparency of the tissue (see Liver). The readiness with which the change may be detected is dependent upon the degree to which the organ is affected. The beginning process is most easily detected when associated with fatty degeneration of the parenchyma, in which case the yellow areas stand out plainly in the transparent tissue. The vascular apparatus of the organ is first affected, especially the capillaries of the glomeruli, which may be alone involved. When this is the case, examination of the cut surface of the organ, after treatment with Lugol's solution, discloses numerous small dark-brown bodies disseminated through the cortex.

The methods of demonstration and examination of the affected tissue are the same as those given for this change in the liver, and may be applied to fresh or hardened sections.

M. F., F. M. stain with Lugol's solution, mount in glycerine or stain with gentian violet, mount in potassium acetate or Zenker, celloidine. Mallory's connective tissue stain. (Amyloid blue, epithelium red.)

The amyloid substance appears brown in a yellow background, or rose in a blue background, according to the stain employed.

The glomeruli reveal the first evidence of the amyloid change. This may affect the entire glomerulus or a single loop. (Plate VIII., Fig. 5.) In severe cases the vasa efferentia, the interlobular vessels, or the entire vascular apparatus of the kidney may be affected. Amyloid degeneration of the Art. rectæ may be detected with the unaided vision as fine brown lines in the pyramids. Beside

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the degeneration of the vessels of the medulla, a form of the process affects the tunica propria of the tubules, so that a confusion of the two must be avoided. This difficulty is most likely to arise where the epithelium of the tubule has been desquamated. In moderately advanced stages of the disease, the amyloid deposits produce the most delicate demonstration of the secretion apparatus of the organ. In favorable sections the interlobular arteries, with their dependent glomeruli, stained a deep rose or brown, may be seen, presenting an appearance not inaptly compared to bunches of grapes. Preparations which are to be preserved in balsam are best stained after van Gieson, or with polychrome methylene-blue. Plate VIII., Fig. 6, shows a stage of the process in which the endothelium of the glomerular vessels is still intact, but surrounded and pressed together by an unstained (hæmatoxylin) glistening mass of amyloid substance. Widespread thrombosis in the veins of the kidney commonly accompanies amyloid degeneration of the organ. An investigation of the vessels with this in mind becomes a matter of routine in amyloid disease of the kidney.

14. Deposits in the Kidney.

The relatively great distance which the urine traverses from the glomeruli to the papillæ predisposes to the deposition of certain substances held in solution by the urine. The function of the tubular epithelium appears to be that of excreting the solid constituents of the urine. The urine, therefore, gains in concentration as it flows downward. Exactly what conditions of the urine predispose to deposition of the solids is not determined in full, but concentration undoubtedly plays an important part. The deposits are usually in the epithelium, or lumena of the tubules, or in the connective tissue structure. When the entire tissue is loaded with such a deposit, which occurs in the papillæ, we speak of an infarct.

(*a*) **Uric Acid Deposits.** (*a*) **Kidney of Gout.**—The gouty diathesis commonly leads to inflammatory changes in the kidney, resulting in a form of contracted kidney. The kidney of gout is characterized by the deposits of uric acid scattered through the parenchyma of the organ. These deposits are similar to the deposits of uric acid about the joints. The deposits in the kidney may often be detected with the naked eye, as small, pure white points scattered irregularly over the cut surface of the cortex or medulla. These may be readily differentiated from the characteristic chalk infarcts of the papillæ by their localized character and irregular distribution through the kidney, especially as no deposits of chalk similarly distributed occur in contracted kidney. Certain knowledge of the character of the deposit is, however, only obtained by the microscopic examination, which is best made with *fresh material* obtained by snipping off with the shear the surface of one of the white deposits and examining the tissue thus obtained in salt solution. Moderate pressure may be applied to the cover-glass.

Higher power shows at the point where the white deposit was seen, an aggregation of fine closely approximated needles and plates. These are composed of sodium urate, and are dissolved in acetic acid or hydrochloric acid on the addition of heat. Occasionally crystals, concretions, or needles of chalk are found deposited with the crystals of sodium urate. These likewise dissolve in hydrochloric acid, but their nature may be determined by treatment with dilute sulphuric acid. Sections of the tissue which have been hardened, embedded and stained in the usual way show at the point of deposit (the crystals having been dissolved in the hardening solutions) an area of necrosis surrounded by a zone of reactive round-celled infiltration. This necrosis is produced by the uric acid deposit. If the tissue be hardened in absolute alcohol and stained with an alcoholic solution of vesuvin, it will be found that the crystals have extended into the living tissue and that they are in many cases surrounded by foreign body giant cells.

(*β*) **Uric Acid Infarct.**—Found most commonly in the kidney of infants who have lived but a short time, and may be recognized as yellowish or reddish yellow radiating striations of the papillæ following the course of the collecting tubules.

The examination must be made in the fresh state by snipping off a piece of tissue with curved shears, or the tissue may be hardened in alcohol and cut with the section razor. When exposed to aqueous solutions (and formaline) the uric acid is rapidly dissolved.

Moderate magnification shows the striation of the papillæ to be caused by dark cylindrical masses lying within the lumena of the collecting tubes. If the hand is placed over the mirror of the microscope, thus cutting off the direct illumination and permitting the object to be illuminated from above, these masses again appear red as in the macroscopic preparation. Higher magnification resolves these cylinders into spheres of irregular size. These consist of acid sodium urate and are dissolved in acetic or hydrochloric acid. Uric acid infarct is an extremely rare occurrence in still-born infants, for which reason their presence may be taken as a more or less certain sign of extra-uterine life.

Deposits of uric acid may be examined in hardened and embedded sections by carefully observing the following steps. The material must be hardened in alcohol, embedded in paraffine and fastened to the slide. Remove paraffine with xylol, transfer to absolute alcohol and then place in alcoholic solution of vesuvin (vesuvin 1 gram, absolute alcohol 50 to 100 ccm.), stain 3 to 5 minutes, wash rapidly two or three times in water, dehydrate in absolute alcohol, clear in xylol, mount in Canada balsam. On examination of the section the spheres and cylinders of uric acid are found closely packed within the lumena of the tubes. The epithelium of the tubes is usually present, although not uncommonly flattened by pressure from the closely packed masses of uric acid. This most commonly occurs where two collecting tubules unite. The spheres of uric acid frequently possess a double contour or a distinct radiating structure. A section of the tissue should be stained with hæmatoxylin for purposes of control when it will be found that the uric acid deposits have entirely disappeared. In place of the cylinders of uric acid, hyaline cylinders will be found. These are composed of the hyaline mass which binds the uric acid deposits together. This albuminoid substance is excreted at the same time with the minute spheres of uric acid which are found in the secreting portion of the tubules. When the tissue is stained with van Gieson's stain an albuminoid structure of the spheres of uric acid may be detected by its blue-violet color.

(*b*) **Pigmentation of the Kidney.**—(*α*) Bilirubin infarct or hæmatoidin infarct. The darker the color of the papillary infarcts of the new-born the greater the possibility of the presence of other coloring matter than uric acid. These coloring matters are usually bilirubin or hæmatoidin, and when noted the other organs of the body reveal the presence of a general icterus (icterus neonatorum). The coloring matter of the bile or of the blood itself is carried to the kidney by the circulation and deposited in the epithelium or the interstitial tissue of the papillæ in the form of rhombic crystals or fine needles (bilirubin or hæmatoidin crystals). (Plate X., Fig. 2.)

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Ebstein und Nikolaier. Ueber die Ausscheidung der Harnsäure durch die Nieren. Virchow's Archiv, Bd. 143, 1896.

Aschoff. Verh. d. deutschen path. Gesellschaft, München, 1899.

Fresh sections cut with the shears or freezing microtome sections from material hardened in formaline (carmine) should be examined.

(β) A second form of pigmentation occurs in the kidney as a result of senile atrophy. The pigment is deposited in the superficial layers of the epithelium of the cortex and is associated with a gradual hyaline degeneration of the glomeruli. The surface of the kidney remains smooth or becomes finely granulated. As a result of atrophy of the tubules the glomeruli are found directly beneath the surface of the organ and when these become calcified they appear as fine white bodies scattered over the surface. A great many of the calcified bodies are, however, found to be due to minute cysts, the contents of which have become calcified. The epithelium of the loops and in the neighborhood of the connecting portions of the tubules contain fine granular pigment. As this pigment gives no reaction for iron, it is probably autochthonous.

(γ) Iron-containing pigment is found in kidneys which have been affected by hemorrhagic nephritis, or about the periphery of or within hemorrhagic infarcts of the organ and in the kidneys of individuals who have suffered from diseases in which there has been a great destruction of the red blood corpuscles (pernicious anæmia, malaria). The granules of hæmatogenous pigment are deposited in the epithelium of the convoluted tubules and loops of Henle, and the capsule of Bowman. In rarer cases the endothelium of the capillaries and the connective tissue stroma may contain pigment (hæmosiderosis). In hæmachromatosis the epithelium of the uriniferous tubules from the glomeruli to the point where they enter the collecting tubules may be loaded with iron-containing pigment. In these cases a certain amount of pigment which is free from iron is likewise present. Usually uric acid and bilirubin or hæmatoidin infarcts are found together, but they may occur separately.

Formaline, F. M., carmine.

(δ) **Hæmoglobin Infarct.**—When hæmoglobin or methæmoglobin (poisoning by potassium chlorate) is thrown into solution in the blood, it is secreted through the glomeruli and is commonly deposited in the lower portions of the reniculus. Here it appears in the form of brownish red drops, which may be packed together in the form of cylinders. The epithelium of the straight tubules frequently takes up these drops of hæmoglobin. In such cases the hæmoglobin likewise appears in the urine (hæmoglobinuria). Where hæmoglobin is present, the kidney has a dirty brown or blackish striation in the medulla (macroscopic observation).

Formaline, F. M., carmine.

(ε) **Icterus of the Kidney.**—In general icterus the kidneys possess a diffuse yellow or greenish yellow color and in severe cases the medulla of the organ presents a yellowish brown or dark green, almost black, striation. The parenchyma is generally affected by excessive fatty changes.

Examine fresh double knife sections or harden in formaline, F. M., carmine or sublimate, embed, hæmatoxylin.

Where the uriniferous tubules appear in cross section, green or yellowish homogeneous masses are found to occupy their lumena. These are not uncommonly so extensive as to produce typical cylinders. They consist of a mixture of inspissated bile coloring matter and precipitated albuminous substance from the urine. Granular deposits are found in the epithelium in almost all regions of the tubules. A great many of these granules are nothing more than the intensely stained cell granules of the swollen epithelium (convoluted tubules).

REFERENCE:

Orth. Ueber das Verkommen von Bilirubinkrystallen bei neugeborenen Kindern. Virchow's Archiv, Bd. 63, 1875.

Very probably the epithelial cells have absorbed the soluble coloring matter of the bile. This is either precipitated in the cell in the form of homogeneous granules which are identical in appearance with the so-called cell granula, or that portion of the protoplasm which has taken up the coloring matter is the constituent, which, when precipitated by the hardening reagent, produces the cell granula. Deposits of greenish, granular pigment are found where the epithelium has disintegrated, showing that the coloring matter was intimately combined with the protoplasm. Isolated areas of necrotic epithelium are commonly found where the kidney is affected by icterus. In *fresh double knife sections* the epithelium of the tubules presents a diffuse yellowish coloration.

(*c*) **Chalk Deposits in the Kidney.**—(*α*) **Chalk Infarct.**—Deposits of carbonate of lime in the form of infarcts of the papillæ occur in adults as a result of destructive diseases of the bone, which are associated with marked absorption of lime salts. The apices of the papillæ show a white striation, which, when extreme, may give the papillæ a distinct chalky appearance. Examination is best made by the aid of fresh preparations obtained with the shears, or freezing microtome sections, examined in water. When examined with low power the tissue in the papillæ appears much darker than the surrounding tissue. When illuminated from above this darkening of the tissue changes to a whitish appearance. When examined with high power the dark appearance of the tissue is found to be due to the presence of numberless fine granules of a substance which possesses a high refractive index and which prevents the passage of light through the tissue. These are found in the connective tissue stroma, the tunica propria, the epithelium and, in rare cases, in the lumena of the tubules. They disappear when treated with hydrochloric acid, and are, therefore, not fat droplets, for which they might be mistaken. They may possibly consist of chalk. To determine this the preparation must be treated with dilute H_2SO_4, when the granules disappear and, after a period, rhombic needles of sulphate of lime are formed ($CaSO_4$). The diagnosis of chalk deposit is then established.

(*β*) A second form of chalk deposit occurs in the kidney as a result of sublimate poisoning. In this case the deposit of chalk is preceded by necrosis of the epithelium. Chalk granules are deposited in the necrotic cells. The necrosis and calcification of the epithelium is distributed irregularly through the tissue and principally affects the convoluted tubules.

(*γ*) Lastly, various forms of chalk deposit are found in contracted kidneys. The surface of the contracted kidney is frequently covered with minute white dots. These are usually calcified glomeruli which approach the surface as a result of the widespread atrophy of the tissue. Again, a certain number of these minute calcified masses consist of small cysts. Examination of the chalk deposits in these cases is best made by the aid of fresh double knife sections. The deposits then appear as dark masses in the tissue. The usual reactions to determine the nature of these deposits must be employed. If the tissue is hardened in Müller-formaline solution the greater part of the chalk passes into solution in the fixative. To preserve the chalk the tissue must be hardened in pure formaline or absolute alcohol. The necrotic and calcified epithelium or hyaline groundwork which forms the supporting structure for the chalk deposits stains deeply with hæmatoxylin or alum carmine and may be recognized by this appearance.

(*d*) **Glycogen Deposits in the Kidney.**—Deposition of glycogen in the kidney in diabetes is associated with a most advanced form of parenchymatous degen-

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PLATE IX.

Fig. 1.

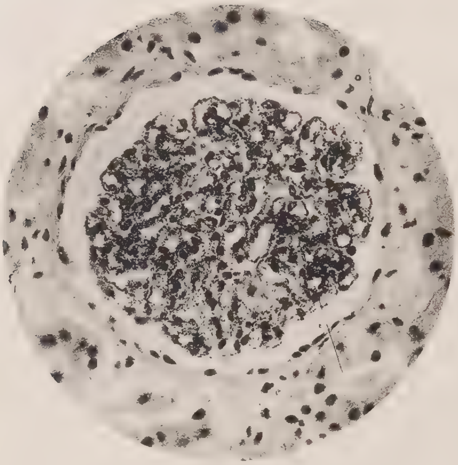


Fig. 2.

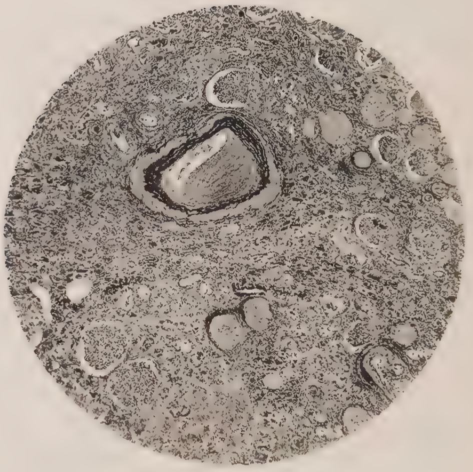


Fig. 3.

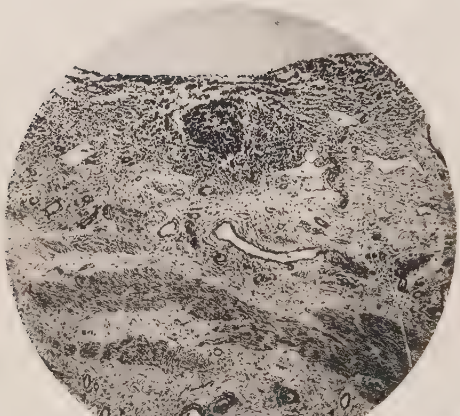


Fig. 4.

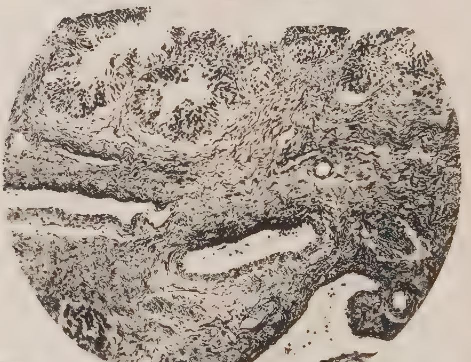


Fig. 5.

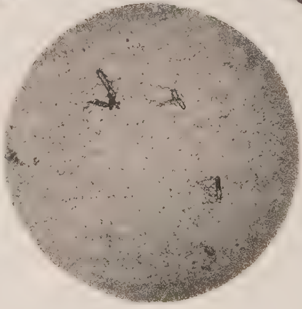


Fig. 6.

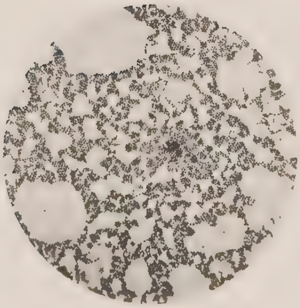


Fig. 7.

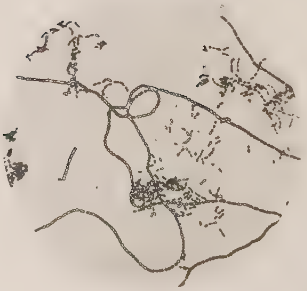
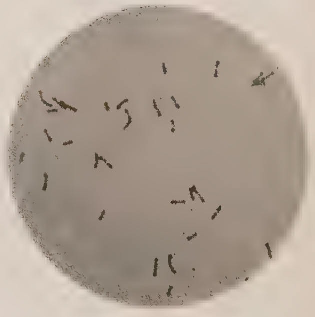


Fig. 8.



eration of the organ. Parenchymatous changes are usually associated with rich deposits of glycogen in the loops of Henle. Fresh double knife sections or sections made from material hardened in alcohol and treated with Lugol's solution take on a diffuse brownish coloration. On examination under the microscope the brown color is found to be localized in the epithelial cells and appears in the usual form in drops of glycogen within the protoplasm. (Plate X., Fig. 1.) As glycogen is soluble in water it is abstracted from tissue hardened by the usual methods, in which case the protoplasm of the cells in hardened sections merely contains large vacuoles.

(*e*) **Renal Cylinders or Tube Casts.**—All substances of sufficient consistence, after excretion into the renal tubules, take on a cylindrical form, which, under certain conditions, they may retain, and are then known as renal cylinders.

These are practically casts of the lumena of the tubules. (Plate VIII., Fig. 1.) Such casts may consist of blood globules, compressed pus cells, masses of bacteria, epithelial detritus, masses of colloid material resulting from degeneration of desquamated epithelium, excreted and inspissated bile or blood coloring

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Ehrlich. Zeit. f. klin. Med., VI., 1883, p. 33.

DESCRIPTION OF PLATE IX.

FIG. 1. Argyria of the kidney. High dry power. Hæmatoxylin. Kidney from case of poisoning with silver nitrate. At the center a glomerulus in the walls of which may be seen (*magnifier*) fine black granules. These are deposits of metallic silver in the walls of the capillaries.

FIG. 2. Arterio-sclerotic contracted kidney. See p. 117. Low power. Hæmatoxylin orcein. Above and slightly to the left an oblique section of enlarged vessel, in which the elastic layer appears as a black, irregular ring. Within this the more poorly stained intima encroaches upon the lumen leaving but a narrow oval slit, within which a few red blood corpuscles may be seen (*magnifier*). The excessive thickening to the right is partially due to the obliqueness of the section. The intima is distinctly thickened. To the right the glomeruli are closely packed and mostly hyaline. The stroma of the kidney is markedly thickened and the tubules are markedly compressed. To the right and beneath a sclerotic vessel. To the left two hypertrophic glomeruli. Near the center two hyaline glomeruli surrounded by a thick wall of newly formed elastic fibers.

FIG. 3. Cystitis nodularis. See p. 127. Middle power. Hæmatoxylin. Above, the surface of the bladder. Running through the structure are bands of muscle fibers. Near the center an empty blood vessel. The epithelium of the bladder is missing (post-mortem desquamation). In the middle directly beneath the surface a dark lymphatic nodule. The adjacent mucous membrane is thickly infiltrated with cells (*magnifier*).

FIG. 4. Cystitis glandularis. See p. 127. Middle power. Hæmatoxylin. Above the superficial surface of the mucous membrane. To the left epithelial nests extending into the bladder wall (v. Brunn). The connective tissue stroma of the bladder wall is greatly thickened. Three dilated and empty vessels are seen.

FIG. 5. Typhoid bacilli with flagellæ. Oil immersion. Loeffler's flagellæ stain. Four comparatively large rods from which extend fine wavy threads (flagellæ). (Agar culture.)

FIG. 6. Staphylococcus aureus. Pure culture. Oil immersion. Gentian-violet. With the magnifier the organisms are seen to be minute deeply stained spherical bodies of regular size.

FIG. 7. Streptococcus. Pure culture. Oil immersion. Gentian-violet. Long chains of spherical and oval organisms. To the right are a large number of organisms in pairs which present an appearance exactly like the diplococcus pneumoniæ (Fraenckel).

FIG. 8. Diphtheria bacillus. Culture. Oil immersion. Thionin. Slender slightly curved rods with enlarged deeply stained ends.

matter (icterus hæmoglobinæmia), fibrin and, lastly, the usual hyaline cylinders, consisting of transparent albuminoid substance, which is, no doubt, excreted in soluble form from the blood and coagulated in the urine. These hyaline cylinders may contain fat droplets or epithelial cells which show marked fatty degenerative changes. In uric acid and chalk cylinders these are held together by an albuminous groundwork, which may be rendered visible by dissolving out the chalk or uric acid.

The fresh examination of the solid constituents of urine is readily carried out by either sedimenting or centrifuging the fluid under examination. The urine may contain squamous epithelium (from the external orifice), pus corpuscles and epithelium from the conducting portion of the urinary tract. These last vary greatly in form and size. They may be either cylindrical or rounded in appearance. A differentiation between the epithelium of the bladder and the pelvis of the kidney, although morphological differences are given in various text-books, is impossible.

The sediment in the urine may be stained by making a cover-glass preparation and fixing it in 10-per-cent. formaline solution. After rapid washing in water this may be stained in Sudan III. (concentrated solution in 70-per-cent. alcohol) for about ten minutes. Wash in 70-per-cent. alcohol, counterstain in hæmatoxylin or alum carmine.

In the investigation of urinary sediment for tubercle bacilli it is necessary to differentiate between true tubercle bacilli and the smegma bacillus. The smegma bacillus is decolorized somewhat more readily than the tubercle bacillus, by which means it may be differentiated from it, although there are certain exceptions to this rule. A good method has likewise been recommended by Marzinowsky.

Stain cover-glass preparations of the sediment with carbol fuchsin three to five minutes. Wash thoroughly in water. Counterstain with Loeffler's methylene-blue two to three minutes. The tubercle bacilli are not stained. The smegma bacilli are red or where staining with methylene-blue is continued they take on a violet color.

15. Cystitis.

The chemical and bacterial irritants which are excreted by the kidney likewise influence the remainder of the urinary tract (kidney, pelvis, ureter and bladder). We have already seen how an infection starting low down in the tract may produce an ascending inflammatory process which may ultimately reach the kidney. (Fig. 16.) In proportion to the intensity and duration of the irritation the cystitis, ureteritis or pyelitis, may be mild or severe. The simple, acute and tuberculous forms are identical with those of all mucous membranes, and the student is referred to the descriptions given under that heading. Only the chronic forms of cystitis present peculiarites worthy of detailed description. In these cases the mucous surface of the bladder is studded with minute whitish

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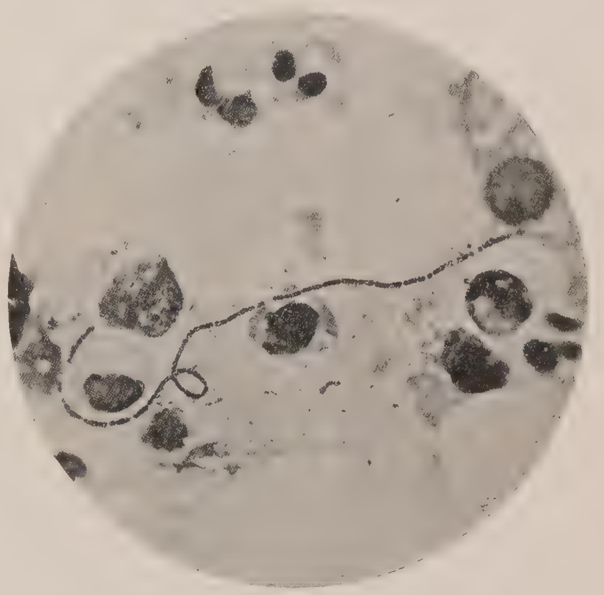
Salkowski und Leube. Die Lehre vom Harn., Berlin (Hirschwald), 1882.
Neubauer u. Vogel. Analyse des Harns, Wiesbaden (Kreidel's Verlag), 1898.
M. Cohn. Zeitschr. f. klin. Med., Bd. 38.
Centralbl. f. Bakt., Bd. 26, 1899.
Cowie. A preliminary Report on Acid-resisting Bacilli in the Lower Animals. Journal of Experim. Medicine, Vol. V., No. 2.

points similar in appearance to tubercles, but which under the microscope present marked differences.

(*a*) **Cystitis Glandularis Cystica.**—The mucous membrane of the urinary tract from the kidney to the external orifice of the urethra never contains, in young and healthy individuals, glandular structures of any sort. The epithelium lining this tract consists of several layers of flattened epithelium, the surface of which is perfectly smooth except that it is thrown into folds by septi of connective tissue, which lie directly beneath the surface. In older individuals, nests of epithelium, first described by van Brunn, may be found embedded in the surface of the epithelial covering of the bladder. (Plate IX., Fig. 4.) These consist of solid nests, and are produced by excessive prominence of the connective tissue septi, which, in many cases, become adherent to each other, and invaginate and isolate portions of the epithelial covering. This agglutination of the adjacent connective tissue septi is exceedingly prone to occur in cases of cystitis. F., F. M., N. S.

Under low power, beside nests of solid epithelium, gland-like structures or tubes may be found, which apparently penetrate deeply into the muscular wall. Between these more or less extensive tubes and the small nests of epithelium a variety of intermediate forms may be found. Under high power the larger of these glandular structures are found to possess definite lumena and double layers of cylindrical epithelium. If the openings of these folds of epithelium be closed, they lead to the formation of minute cysts, which are filled with a clear fluid, and may be detected upon the surface of the bladder, or in other portions of the urinary tract. They are usually located around the openings of the ureters in the bladder, in the pelvis of the kidney and in the neighborhood of the external orifice of the urethra. These glandular, vesicular formations are unquestionably due to chronic inflammatory irritation, as they are surrounded by a more or less diffuse round-cell infiltration.

Fig. 16.



Streptococcus in pus from bladder of case of purulent cystitis. (Oil immersion.)

(*b*) **Cystitis Nodularis.**—F., F. M., N. S. In other cases of chronic cystitis the minute nodes which are detected upon the surface are of an entirely different character. These do not consist of invaginated nests of epithelium, but of numerous lymphoid nodules in the superficial layers of the mucous membrane. (Plate IX., Fig. 3.) These lie directly beneath the epithelium, and cause small protrusions of the surface. They consist of closely packed masses of lymphocytes, and are either newly formed lymphoid nodules or represent an inflammatory enlargement of preëxisting nodules.

Reference:

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V. PANCREAS.

Structure.—The pancreas is a tubular acinous gland, and is made up of numerous lobules. These are supported by an interstitial connective tissue structure, which includes a moderate amount of fat. The pancreas may be differentiated from the parotid gland, which it closely resembles, by the so-called Langerhans cell islets. These consist of small, irregularly polygonal epithelial cells, with round nuclei disseminated singly or accumulated in groups within the lobules and have no definite arrangement about *a lumen.* They have the same origin as the acini but are independent of them, and appear to have a close relation to the vascular system of the organ. These cell islets are nearly three and one-half times as numerous in the tail of the pancreas as in the other region of the organ (Opie).

A simple form of atrophy of the pancreas has been observed which is associated with infiltration of the connective tissue stroma of the organ by fat tissue. In the syphilitic new-born the pancreas is occasionally enlarged and of increased consistence. Examined microscopically, the unusual density of the organ is seen to be due to a marked increase in the connective tissue stroma, which is likewise infiltrated with round cells. Connective tissue surrounds and may penetrate into the lobules. The lumenæ of the glands are frequently surrounded by the connective tissue and the cells of Langerhans are displaced, being found only in the neighborhood of the vessels.[1]

Lesions of the islets of Langerhans appear to bear some relation to diabetes. Opie recognizes interlobular and interacinous chronic interstitial pancreatitis. In the former the process begins at the periphery of the lobules and the islets of Langerhans are affected only in the advanced stage of the disease. In the interacinous form the islets of Langerhans are invaded and broken up. In one case of the interlobular form, in which the islets were affected, mild diabetes was present, and two of three cases of the interacinous form were associated with diabetes. In one of the cases and in a case subsequently reported, Opie found hyaline degeneration of the islets of Langerhans. As destruction of the pancreas in both man and animals is associated with diabetes and as diabetes is absent when serious changes are induced in the secreting portions of the gland by obstruction of the pancreatic duct (the islets of Langerhans are not involved in the obstructive changes), he concludes that the observations which have associated the pancreas and diabetes, are due to the evident relation between diabetes and the islets of Langerhans. Fig. 17 is from a case of diabetes and shows a well-defined ring of connective tissue surrounding an islet of Langerhans. This was the condition throughout the entire organ. The sclerosis was localized to the connective tissue immediately surrounding the islets. (Case of Dr. Betts.)

A very characteristic change which occurs within and in the neighborhood of the pancreas is the so-called fat necrosis. This is associated with various diseases of the organ, such as rupture, carcinoma, hemorrhage and hemorrhagic pancreatitis (Fitz). The areas of necrosis may be very small, from scarcely visible specks to small foci the size of a pinhead. They are opaque in appearance and may be distinctly felt by their increased consistence. Examined in fresh sections, the foci may be detected by their dark brownish appearance. Within the area the normal fat cells have disappeared and the tissue is permeated with minute fat droplets and fine needles which are commonly arranged in thick bundles around the peripheries of the remaining cells (fatty acid crystals). At the periphery of the focus is an accumulation of fat droplets. Except in very recent cases the fat droplets and crystals of fatty acids are replaced by shining masses and clumps which are not affected by the ordinary reagents. When, however, they are treated with H_2SO_4 they are dissolved and

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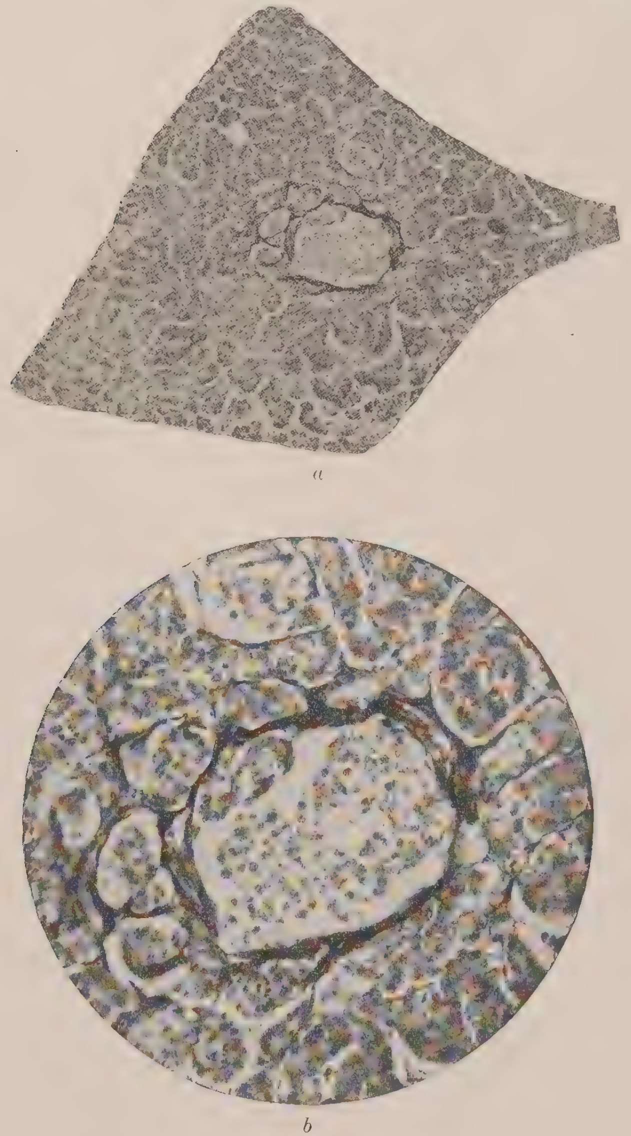
[1] Ueber angeborene Syphilis, see Hecker, D., Archiv f. klin. Med., Bd. 61, 1898. And Opie on the relation of chronic interstitial pancreatitis to the islands of Langerhans, Journal of Experimental Medicine, Vol. V., Nos. 4 and 5.

FIG. 17.



a. An acinus from pancreas of case of diabetes. Near the center a cell islet of Langerhans surrounded by a sclerotic capsule of connective tissue (L. P.). *b*. Same islet by higher magnification. Van Gieson's stain.

are replaced by calcium sulphate crystals. The masses are, therefore, calcium salts of fatty acids.

F., F. M., or celloidin. Hæmatoxylin and van Gieson.

In hardened sections the foci are found to possess a more or less homogeneous structure. The necrosis may affect but a single fat cell or may extend over one or more lobules. Under high power the fat cells are found to have lost their nuclear stain and the contents of the cells are likewise altered. In the smaller foci the boundaries of the fat cells may occasionally be made out. These possess a wide palely stained border which is apparently composed of coagulated albumin. The center of the cell is apparently empty. The degeneration products of the fat have been dissolved out by the alcohol and ether. Occasionally the boundaries between the cells are broken down and the homogeneous material surrounding them becomes confluent. Not infrequently deep blue masses will be found within the foci and when present are due to the combination of degeneration products with calcium. The peripheries of the different foci present different appearances. Occasionally the boundary is entirely free from inflammatory reaction and again marked round-celled infiltration and coagulated fibrin may be found surrounding the degenerated tissue. Occasionally the periphery of the necrotic area contains crystallized blood pigment. This necrosis and alteration of the fat tissue is attributed to the action of the pancreatic secretion which contains a fat-splitting ferment.

Chiari describes a form of necrosis of the pancreas which he attributes to intravital action of the pancreatic juice, in which the necrotic areas are surrounded by active inflammatory changes. Very probably arterio-sclerosis is a factor in the production of these necrotic foci. A certain number of them, however, may be produced by hemorrhage into the pancreas. This form of necrosis of the pancreas is comparatively rare and it is of interest to note that the areas of necrosis are located in the central portion of the pancreas which lies directly over the spinal column, and which is more or less exposed to pressure.

VI. LUNG.

Structure.—The structure of the air passages is more or less characteristic and serves as a ready means of identification. They are lined with a mucous membrane, the surface of which is covered with a layer of ciliated epithelium. In the deeper portions of the membrane is a rich supply of mucous glands, surrounded by a structure made up of smooth muscle fibers, connective and elastic tissue, and including more or less perfected rings of cartilage. The general structure of the mucous membrane lining the nasal cavities, larynx, trachea and bronchi conform to this arrangement, which ceases only with the respiratory parenchyma proper. The terminal bronchi no longer contain cartilage in their walls, the lining epithelium possesses no cilia, and is low cylindrical, or even squamous in form. The terminal bronchi are divided into two or three branches, from the sides of which spring the air vesicles. These are known as alveolar passages. They still contain a certain number of smooth muscle fibers in their walls, which are arranged in rings about the openings of the air vesicles. The air vesicles, or alveoli, contain no muscle fibers. Their walls are made up of a moderate amount of connective tissue and a large amount of elastic fibers, which support a very rich capillary system. They are lined within with a single layer of flattened epithelium and large plates of protoplasm which contain no nuclei. The air vesicles and alveolar passages which spring from a single bronchiole are included together and are called an acinus. A large number of these acini are usually included between thick septi, and are known as a pulmonary lobule. The larger bronchi and the blood and lymph vessels traverse the connective tissue septi which separate the lung into lobules, each of which receives its supply of air by a separate bronchus and its blood and lymph by separate vessels.

The lung contains two separate vascular systems, one serving for the nutrition of the pulmonary tissue, springs from the bronchial artery and terminates in the bron-

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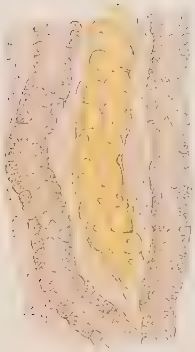
PLATE X.

1.



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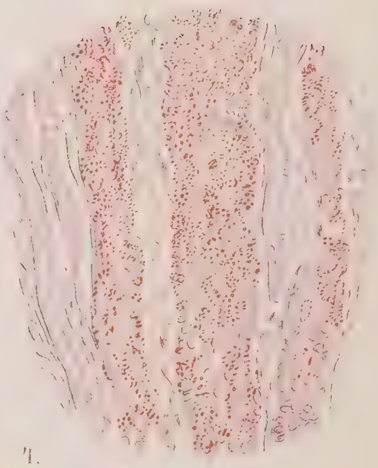

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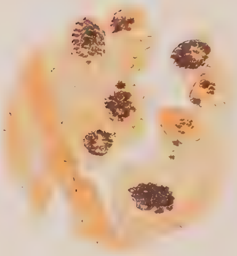

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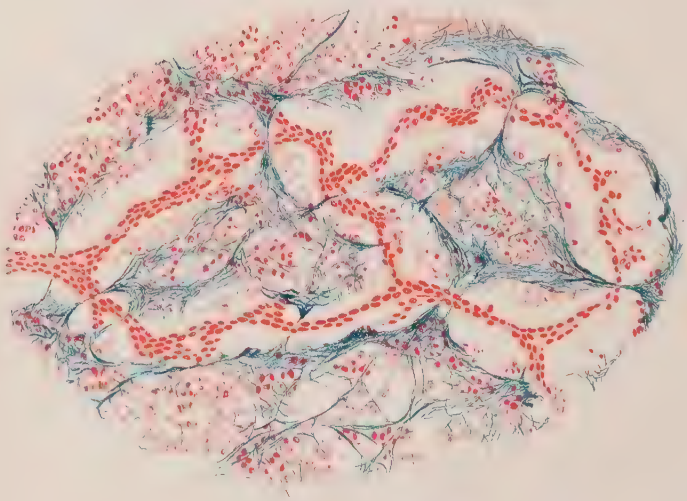

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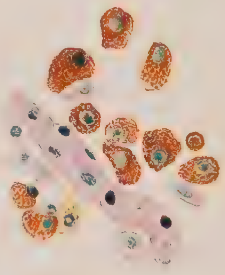

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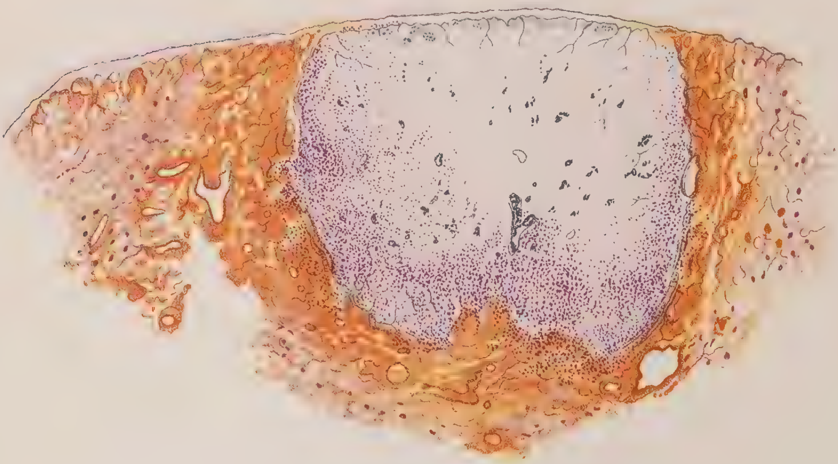

chial veins. The other consists of branches of the pulmonary artery and vein, which, through the medium of their terminal capillaries, permit of the exchange of gases between the blood and the inspired air. The terminal bronchi, alveolar passages and alveoli springing from a single bronchus do not lie together, the structures arising from the various bronchi crossing each other. Therefore, the inflammatory changes spreading from a bronchus do not form compact areas, but appear as disseminated groups of diseased vesicles.

In the microscopic examination of the lung the following structures must be examined: alveoli (contents, diameter, walls); bronchi (contents and walls); vessels (especially the contents); connective tissue septi.

1. Chronic Cyanosis of the Lung.

As a result of various derangements of the cardiac function, but more commonly due to injury of the mitral valve, the blood stream in the lung becomes weak and slowed, with the result that a state of chronic engorgement of the pulmonary capillaries ensues. The lung appears engorged, and on section is of deep red color; in advanced cases deep brown (brown induration).

The microscopic investigation should be begun by examining in salt solution the juice that may be obtained by passing a knife over the cut surface of the organ. Beside larger and smaller fragments of lung tissue, a great number of cells will be seen of about the diameter of the alveolar epithelium (occasionally larger) the protoplasm of which is loaded with brown pigment. These are the characteristic cells found in the lungs of patients suffering from chronic heart lesions. They may likewise be observed in the sputum of such patients and are known as the pigment-cells of chronic heart disease (Herzfehlerzellen). If a portion of the pulmonary tissue be snipped off with a curved shear and pressed out under a cover-glass and examined with low power, the pulmonary capillaries will be found to be excessively engorged. The convolutions in the capillaries are greater than normal and protrude to an unwonted degree into the lumenæ of the air vesicles. The distribution of the pigment in the tissue can only be properly studied in hardened sections.

F. M., embed in celloidin. Stain with carmine. The tissue to be hardened should be selected from portions of the lung which have not been ex-

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DESCRIPTION OF PLATE X.

FIG. 1. Infiltration of the kidney epithelium with glycogen in diabetes. See p. 125. High power. Iodine stain. The infiltrated epithelial cells are stained yellow.

FIG. 2. Hæmatoidin infarct of the kidney. See p. 122. Middle power. Alum carmine. A large number of minute brown crystals between the epithelial cells of the straight tubules.

FIG. 3. Dust cells in the lung. See p. 149. Middle power. Alum carmine. Large desquamated epithelial cells containing black pigment (dust). To the left the wall of an alveolus. The alveolar spaces filled with blood corpuscles.

FIG. 4. Fibrinous pneumonia. See p. 138. Middle power. Alum carmine. Weigert's fibrin stain. Fibrin stained blue. Anastomosis of the masses of fibrin through the stomata in the alveolar walls is plainly visible.

FIG. 5. Pigment cells in cyanosis of the lung. See p. 132. Middle power. Alum carmine. Large desquamated epithelial cells containing brown pigment (Herzfehler zellen).

FIG. 6. Septic infarct of the lung. See p. 134. Low power. Alum carmine. Orcein. Gram. At the center extending to the periphery an area of necrosis. The blue masses beneath the elastica and in the necrotic area are masses of organisms. The branching mass near the center occupies a capillary. About the periphery of the necrotic area, purulent infiltration. To the left the infarct is bounded by an interalveolar septum. Surrounding the entire infarct a well-defined hemorrhagic zone.

posed to pressure. The alveoli when examined under low power, instead of being empty as in the normal lung, are found to contain brown masses which conform more or less in size and shape to the alveolar epithelium. Many of the alveoli are completely filled with these groups of pigment. Similar masses may likewise be found in the connective tissue structure, especially in the wider septi. The marked contortion and protrusion of the capillaries into the alveoli is likewise distinctly evident. Under high power the capillaries are seen to be filled with closely packed red blood corpuscles. In certain of the alveoli free red blood globules are found, which indicate that the tension upon the capillary walls has been too great, and that they have finally ruptured. Careful examination of the masses of brown pigment show that the pigment is deposited in cells. (Plate X., Fig. 5.) These are occasionally polymorphonuclear leucocytes, but for the greater part very closely resemble the epithelium of the alveoli. The pigment consists of fine granules and plates which are derived from the disintegration of the red blood corpuscles due to the constant cyanosis. Occasional cells contain more or less perfectly preserved red blood corpuscles. The alveolar epithelium which is still attached to the walls of the alveoli is not uncommonly pigmented. In the connective tissue stroma of the interalveolar septi, many of the connective tissue cells contain pigment deposits. The process by which this pigment is deposited is comparatively simple. Red blood corpuscles are extravasated into the alveolar spaces, rapidly disintegrate and are taken up by the leucocytes, and the epithelium lining the air vesicle. Later the pigment is either deposited in the lymph spaces or is transported by the leucocytes through the lymph spaces and deposited in the connective tissue. This transportation of pigment occurs through minute openings between the epithelial cells lining the alveolar lumen (stomata). In the connective tissue stroma the deposits of pigment are found to conform very closely to the shape of the connective tissue cells and careful examination of these groups of pigment granules commonly discloses the presence of a nucleus among them. The greater portion of the pigment deposited in the lung in cyanosis gives a positive reaction when tested for iron. The longer the cyanosis and deposition of pigment continues, the greater the irritation of the connective tissue and the greater the thickening of the interalveolar walls. The larger septi in these cases are not only thickened but contain more nuclei than normal. A similar condition results whenever pigment is deposited, as in anthracosis.

In lungs affected by pigment deposit certain bodies are frequently found which are of amyloid character but possess a fine radiating and concentric striation (corpora amylacea). These masses lie in the alveolar spaces and occasionally contain a definite brown or black nucleus. Where coal is deposited in the lungs this nucleus consists of coal dust. Where the nucleus is brown it is composed of pigment. If iodine be applied to these bodies, which must be in the fresh state, by treating a small portion of the tissue with Lugol's solution, they take on a deep brown color. A special reaction for the corpora amylacea may likewise be obtained in hardened sections. The paraffine must be removed from the section, pass through absolute alcohol and place in 10-per-cent. hydrochloric acid. Rinse in water, embed in glycerine. The corpora amylacea appear either brown, violet or blue.

In very rare cases of excessive slowing of the blood current in the pulmonary circulation, the blood in certain portions of the capillary system may come to a complete standstill (stasis). Where this occurs the red blood corpuscles rapidly

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disintegrate and the capillaries are occluded with granules and platelets of pigment.

2. Hemorrhagic Infarct of the Lung.

The occlusion by an embolus of a branch of the pulmonary artery produces anæmia of the wedge-shaped area of lung tissue which it supplies (ischæmia). If the circulation in the lung be normal, the ischæmia thus produced is rapidly relieved by reëstablishment of the blood current through the numerous anastomoses of the capillaries. If the pulmonary circulation be weakened, as in various derangements of the heart, the ischæmia is followed by excessive engorgement of the capillaries of the infarcted tissue, causing rupture of the capillary walls and hemorrhage into the air vesicles. When this results a so-called hemorrhagic infarct is formed. Hemorrhagic infarcts are roughly conical in form, having the base at the pleural surface of the lung, the apex near the occluded vessel. They are of solid consistence, produce elevation of the pleura (caused by contraction of the elastic structure in the walls of the adjacent air-containing vesicles), present on section a smooth surface of deep red color, and when limited by pulmonary septi have sharply defined boundaries.

The infarcted areas are variable in size. The smaller are best suited for microscopic investigation, affording a better view of the entire process. From the larger infarcts pieces of tissue which include the boundary between the infarcted and adjacent lung tissue and the pleura afford the best demonstration.

M. F., F. M. or celloidin embedding. Carmine and elastic fiber stain. F. or F. A. or embed. Fibrin stain.

A general view of the preparation with low power discloses the infarct, which may be readily recognized, the air vesicles within the infarcted area being filled with blood, whereas those of the surrounding tissue are entirely empty, or contain but a few cellular elements. By this magnification the boundary between the infarcted area and surrounding tissue is quite sharply defined, especially when one side of the infarct is delimited by one of the larger pulmonary septi. (Plate XI.) Careful search usually discloses the thrombosed vessel, which is located near the margin of the infarct. (Plate XI.) The thrombus should be recognized by the striation and by layers of blood platelets (see Thrombus). The bronchus which accompanies the artery usually reveals no change. Some authorities maintain that hemorrhagic infarcts in the lung are produced by partial occlusion of the bronchi which supply the infarcted area, attributing the partial occlusion to hemorrhages beneath the mucous membrane, which result from the chronic engorgement of the bronchial capillaries. That this is not the case may be seen in favorable sections, where a bronchus may occasionally be encountered which lies directly on the edge of the infarcted area, in which case the capillaries in the bronchial wall toward the infarct will be found to be markedly engorged, while those on the opposite side of the bronchus show no congestive changes. The veins which spring from the infarcted area usually contain thrombi, an indication that the process of thrombosis has spread from the engorged tissue of the infarct into the efferent veins. Higher powers show that the alveoli are filled with red blood globules which (Plate XII., Fig. 5), if the infarct be of recent origin, are but slightly altered in appearance. In recent infarcts the cells forming the alveolar septi take the nuclear stain. The capillaries, when it is possible to demonstrate them, are tortuous and engorged. The connective tissue stroma and the alveoli contain a large number of pigmented cells. These are most easily seen at the periphery of the infarct and in the adjacent pulmonary tissue, where they are not obscured by the surrounding blood. The presence of these pigmented cells and the engorgement and tortuous appearance of the capillaries indicate long-standing congestion. The hyperæmia is greatest at the periphery of the infarct, where it forms a zone similar to the hyperæmic zone surrounding the anæmic infarct of the kidney. This zone likewise extends across the base of the infarct as a narrow band of hyperæmia in the deeper layers of the pleura. As the infarct increases in age numerous changes appear. As the nourishment is cut off the tissue loses vitality and then perishes. The nuclei no longer take the stain, although the elastica of the alveolar walls remains unaltered for a greater period of time. The blood corpuscles within the alveoli disintegrate, liberating granules of brown pigment, which are absorbed or remain as masses of pigment in the alveoli. At the periphery a zone of granulation tissue composed of spindle and round cells and young capillaries is formed, which begins to invade the infarcted area, and through a process of absorption and organization removes and replaces the necrosed tissue and detritus. The remaining changes in the infarcted tissue are those which occur in the development and degeneration of the connective tissue by which it is replaced. Such tissue has a marked tendency to contract producing marked depressions of the pleural surface.

3. Septic Infarct of the Lung.

It has been stated under the appropriate heading that simple hemorrhagic infarction results from the occlusion of a pulmonary artery by an embolus only when associated with chronic cyanosis. Hemorrhagic infarcts of a different character occur without stasis when the embolus contains infectious material. (Cocci, bacteria, etc.)

The poisons elaborated by the organisms pass into the infarcted area and so injure the walls of the capillaries that the blood from the surrounding tissue flows into the ischæmic area, producing a hemorrhagic infarct.

In describing the septic invasion of the kidney, we have already noted that organisms which find their way into the lymph spaces and then into the venous system, or directly from open wounds into the venous system, first find lodgment in the lungs.

Therefore, when general infection is suspected, the lungs should be the first organs examined for septic emboli.

The examination may be rapidly and satisfactorily carried out by means of sections made with the section razor from pieces of tissue hardened in alcohol. After clearing with potassium hydrate or acetic acid the emboli may be easily identified. (See Kidney.) Permanent mounts are best made from tissue hardened in F., cut on the freezing microtome or embedded, and stained after Gram's method. Previous staining with carmine.

In early pyæmia the lung may show no macroscopic change. When the infection reaches the larger veins (which may take place through the skin or the larger viscera, as the uterus), septic thrombi are formed. These thrombi usually contain large numbers of bacteria. When fragments of such thrombi become

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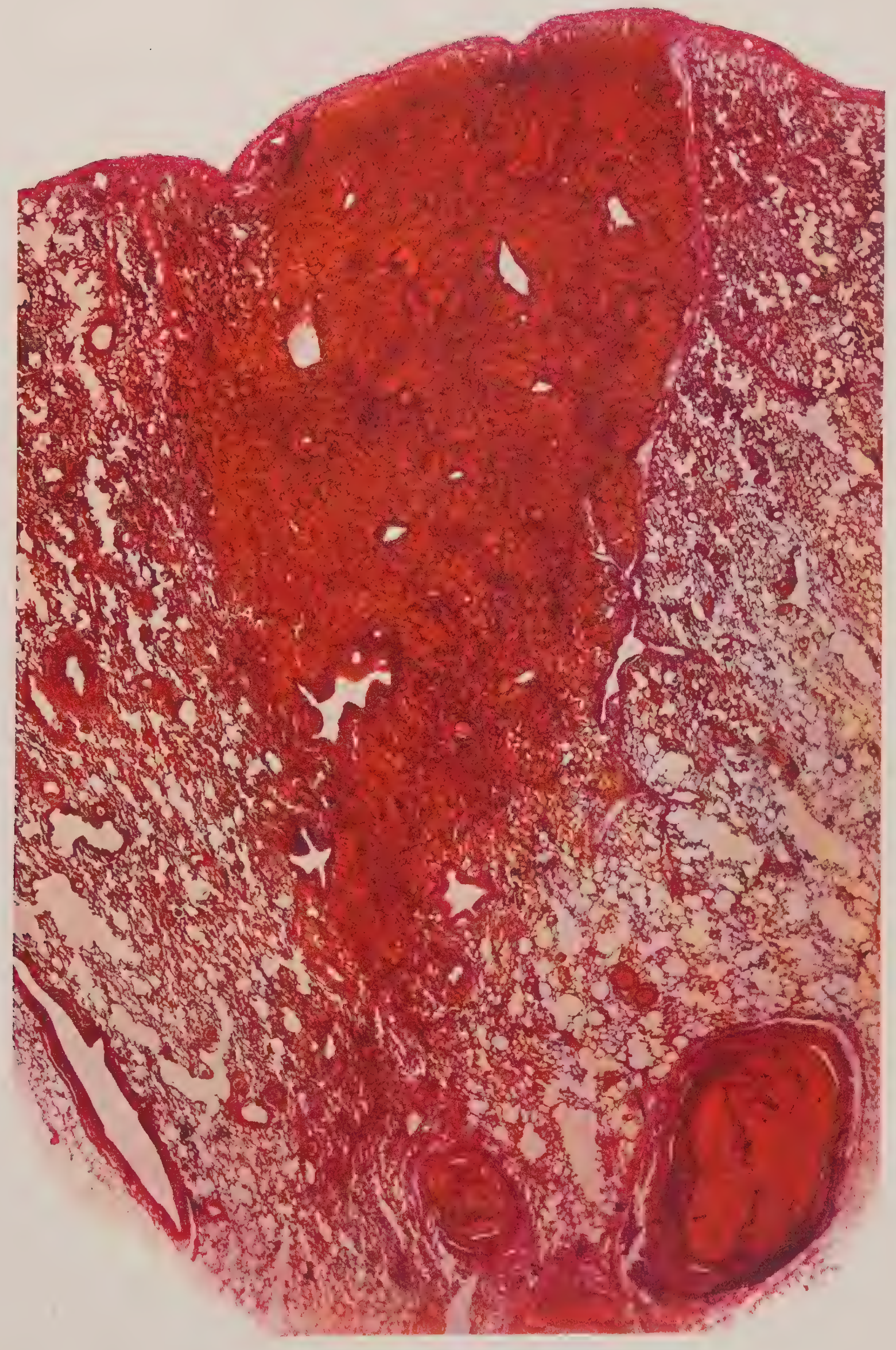
DESCRIPTION OF PLATE XI.

Hemorrhagic infarct of the lung. See p. 133. Tricolor photomicrograph. Very low power (micro-collinear). Hæmatoxylin eosin. The wedge-shaped infarct is stained a bright red. The outlines of the alveoli may be seen, the alveolar spaces filled with blood. Several large empty vessels in the infarct. To the right the infarct is delimited by a pulmonary septum. At the right and left a slightly œdematous air containing parenchyma. Beneath two large vessels containing striated thrombi. The smaller of these to the left is the occluded vessel of the infarct.

PLATE XI.



detached they are carried through the venous system to the lungs, where they occlude vessels of larger size and produce so-called septic infarcts. Septic infarcts differ from the hemorrhagic form in many respects. On inspection of the gross lesion a well-defined yellow (purulent) zone may be made out at the periphery of the infarcted area, or the greater part of the infarct may be composed of a softened, yellow (purulent) mass. A marked hemorrhagic area surrounds the purulent zone and occupies a position between it and the pulmonary tissue. The pleural aspect of the infarct presents a yellowish, discolored appearance surrounded by a hemorrhagic border. The pleural surface of the infarct is usually free from fibrin, while the adjacent pleura, owing to the inflammatory processes which surround the infarct, are covered by a more or less well-developed fibrin deposit.

F., F. M. or celloidin. Carmine or elastic tissue stain, or F. A., F. M. or embed. Fibrin stain.

Low-power inspection of the infarcted area shows practically the same alterations of the tissue as those described under septic infarct of the kidney. (See Kidney.) The tissue included in the infarct is necrotic. Scattered through the necrosed tissue are minute deep blue dots (Plate X., Fig. 6), which, under higher magnification, are resolved into masses of bacteria, lying within the lumena of the smaller vessels. These are detached particles of the original embolus, which have been carried into the infarcted area at the time when the mass became lodged in the principal vessel. The result of this invasion of the infarcted area is seen in the necrotic state of the tissue, which includes the pleural covering. Total necrosis of the pulmonary tissue and pleura prevents the leakage of the fibrin-forming fluids upon the pleural surface of the infarcted area, which accounts for the lack of fibrin in this region.

Higher magnification shows the yellow zone at the periphery to be composed of pus cells (polynuclear leucocytes), which mark the boundary between necrosed and living tissue. The outer red zone, as in the kidney, is due to hyperæmia and hemorrhage into the tissue. If the vessel occluded is small the infarcted area is likewise small in extent, and may receive sufficient blood supply from the surrounding arterioles to prevent necrosis. In this case the collection of leucocytes about the focus leads to the formation of typical miliary abscesses, which differ in no way from those described as occurring in the kidney. If the specific action of the bacteria is sufficient the tissue adjacent becomes necrotic. This necrosis is distinct from the necrosis produced by occlusion of the blood supply in the larger infarcts, and is due to the direct action upon the tissues of the poisons elaborated by the bacteria.

4. Œdema of the Lungs.

When the fluid constituents of the blood alone, or with but few of the corpuscular elements, find their way through the capillary walls into the alveoli, we speak of œdema of the lung. The transudation of fluid through the capillary walls is due to injury of the endothelial continuity produced either by bacteria and their products, chemical irritation, or by stasis of the blood stream, with increased intercapillary pressure.

Section of the lung shows a marked amount of frothy exudate in the bronchi. The lung tissue is heavier and of greater density than normal, does not crepitate when incised, and an abundance of pale red fluid flows from the cut surface.

In order to prepare sections of the tissue, which shall contain the fluid, the methods recommended for albuminuric kidney are employed. Small pieces of the fresh lung are thrown for a few seconds into boiling water, or the tissue may be hardened in formaline which coagulates the albuminous fluid. F. M., N. S.

Under low power the alveoli are found to be filled with a homogeneous substance which is usually separated from the alveolar walls by a narrow space. This space is the result of the contraction of the fluid and the mass is the coagulated albuminous fluid contents of the alveolus. Higher magnification shows that the mass is not truly homogeneous, but contains fine granules of precipitated albumin, and a certain number of red blood corpuscles, leucocytes and desquamated epithelium. (Plate XII., Fig. 4.) In the inflammatory form of œdema a large number of polymorphonuclear leucocytes are found in the fluid.

One of the special causes of œdema of the lungs is invasion and occlusion of the lymph spaces by carcinoma (so-called lymph vessel carcinoma of the lung). (Plate XIV., Fig. 5.) Here the unaided vision may detect the carcinomatous lymph vessels as a fine, white network beneath the pleura, and as white lines and dots surrounding and accompanying the larger vessels and bronchi.

Microscopically the tissue presents the most interesting conditions. The deeply stained masses of carcinoma are localized about the blood vessels. They occupy the enormously dilated perivascular lymph spaces and stand out prominently in the pale œdematous tissue. Higher powers show the masses to be composed of large epithelial cells. These are rich in protoplasm and have large, deeply-stained nuclei, which distinguish them from the desquamated epithelium or polynuclear leucocytes of the œdema fluid.

Another form of œdema is found in the healthy portions of lungs affected by severe chronic inflammation. Here the œdema fluid contains certain products of metabolism, which, owing to the derangement of function in the lung are not readily removed (tuberculosis). The portions of tissue thus affected are usually surrounded by tuberculous areas. The œdematous tissue is distinguished from the tuberculous by the distinct alveolar structure, and differs from tissue affected by ordinary œdema by a marked yellow coloration. The nature of the process may be readily understood by examining in salt solution a fresh particle of lung removed with a curved shear. Under low power the alveoli appear to be filled with dark masses and if these be examined under high power, they are seen to consist of closely packed cells which contain large numbers of fat drops of varying size. These cells are mostly desquamated epithelium, possibly some migrating elements. The masses of fat-laden cells explain the peculiar yellow ochre color of the tissue. For more exact investigation frozen sections may be made of tissue hardened in formaline, stained with Sudan III. and mounted in glycerine, or portions of tissue may be hardened in Flemming's mixture, embedded in paraffine and stained with saffranin.

5. Fibrinous Pneumonia.

Fibrinous or lobar pneumonia is one of the more common acute infectious diseases, and is the result of the entrance into the lung of the diplococcus pneumoniæ, or rarely of infection of the lung by other bacteria. The process usually affects one or more lobes of the lung, more rarely parts of lobes, and runs a typical course which is divided into three stages of progression. The immediate reaction of the tissue to the irritation of the organisms takes the form of a typical, acute œdema. Under the continued irritation of the organisms the vessels in the infected portion become engorged (hyperæmia). This increases until fibrin-forming fluid passes into the alveoli and coagulates. The affected lobe now has a marked increased consistence, which is in proportion to the amount and density of the fibrin coagula in the alveoli, and increases until the tissue may reach a stage in which its density is equal to that of liver tissue (red hepatization). On section the hepatized tissue appears of deep red color with dry surfaces. In this stage of the disease the red color of the tissue recedes and is replaced by a gray or grayish yellow appearance. The density and dryness of the structure is more intense and the tissue becomes distinctly brittle (gray hepatization). The cut surface appears coarsely granular from the projecting fibrin coagula within the alveoli. With the beginning of resolution the tissue becomes softer, the granular appearance of the cut surface disappears, the fibrin breaks down and is removed; the alveoli, gradually becoming freed, readmit air and return to their normal appearance and function. The above-described minute masses of fibrin which fill the alveoli may be considered characteristic of lobar pneumonia. These may be obtained for examination by merely passing the blade of the knife over the cut lung surface and examining in salt solution the fluid obtained. The minute structure of the dark masses may not be properly made out with low power and must be examined with high power. Moderate pressure must be made on the cover-glass to produce sufficient thinning of the material. This is now found to consist of a more or less dense network of shining fibers which include irregular granular and cellular masses. Where the clump of fibrin has not been too much broken up the edge of the mass may be found to be drawn out in a pyramidal-like projection and at this point connected with a similar mass. If acetic acid is now added to the preparation the network of fibers clears, indicating the albuminous nature of the deposit. The material under consideration is therefore fibrin and may be distinguished by this reaction from coagulated mucus, which withstands acetic acid. As the network disappears, the cellular elements which it includes become more definite and are seen to contain round or polymorph nuclei. The more advanced the process the greater the amount of fat contained in the cells. During resolution the fresh investigation shows only remains of fibrin, numerous fat droplets, pieces of tissue and albuminous granules, the remains of disintegrated cells and fibrin. The granulated appearance of the cut surface is produced by the contraction of the elastic alveolar walls, which causes the protrusion of the coagula. The finer differences between the various stages of the process may be best observed in hardened sections.

Harden in formaline, F. M. Carmine, elastic fiber stain or Gram or van Gieson, F. A., F. M., or embed. Fibrin stain.

The appearance of the tissue in the first or œdematous stage is identical with that described under œdema of the lungs. Here the number of red blood globules and leucocytes in the alveoli is increased. In the stage of red hepatization the coagulated fibrin within the alveoli explains the increased consistence of the tissue. The alveoli are filled with yellowish masses, the capillaries are markedly engorged. (Plate XII., Fig. 5.) The yellow appearance of the masses within the alveoli is found on examination with higher power to be due to a predominance of red blood corpuscles in the network of fibrin. (Plate XII., Fig. 5.) The hyperæmia has been followed by hemorrhage into the alveolar spaces. The great number of extravasated red blood globules explains the brown color of the so-called "rusty sputum" of pneumonia patients. The masses of fibrin within the alveoli likewise contain a considerable number of leucocytes and desquamated epithelium beside the red blood corpuscles already noted. The relation between the mass of fibrin and the number of leucocytes varies in each case and even in the alveoli; in some the amount of fibrin is great and the number of leucocytes small, in others the reverse is the case. In hardened sections the fibrin mass does not completely fill the alveolar spaces, but is separated from the wall of the air vesicle by a narrow margin which is due to the contraction of the fibrin during the process of hardening. (Plate XII., Fig. 6.) Here and there, however, the mass is definitely attached to the alveolar wall. If sections are now stained after Weigert's fibrin method the fibrin becomes a deep blue and the fibers and network stand out clearly against the background of lung tissue. (Plate X., Fig. 4.) We may now observe that the above-mentioned filaments of fibrin which appear to be attached to the vesicular wall often pass through the septi and become continuous with the network of fibrin filling the adjoining alveolus. This intercommunication of the fibrin masses explains the great resistance which they offer during life to all attempts at removal and affords a most beautiful demonstration of the manner in which the air vesicles communicate by minute openings through the thinner septi. In sections stained with Weigert's fibrin method the large lymph vessels in the alveolar septi are found to contain fibrin deposits. This indicates that the lymph vessels have absorbed the fibrin-forming fluids from the alveoli. The capillaries likewise contain more or less extensive deposits of fibrin. In sections which have been stained with a simple nuclear stain and embedded in glycerine, these masses in the capillaries have a distinct glistening appearance (so-called hyaline capillary thromboses). To what extent these deposits of fibrin within the vessels depend upon intravital changes, or to what extent they are post-mortem occurrences, may not be readily determined. The permeation of pulmonary tissue with the coagulated fibrin masses greatly reduces the elasticity of the organ, and gives it a brittle and fragile character, already described.

Gray hepatization is marked by the disappearance of engorgement of the capillaries, and the predominance of deeply stained leucocytes in the alveolar contents. The red blood globules have disappeared. (Plate XII., Fig. 7.) The coagula within the alveoli are much richer in fibrin than in the earlier stages of the process (Weigert's method). When the process of resolution or healing begins, the contents of the alveoli gradually disintegrate and are removed by expectoration or by absorption through the lymph channels. As the masses are removed the air is readmitted and the pulmonary tissue resumes its function. The leucocytes first show the changes of karyorhexis and then fall victims to the general disintegration.

By collecting the evidence already presented we may see that pneumonia is a process in which there is transudation into the air vessels of fibrin-forming fluids from the capillaries, associated with an active transmigration of leucocytes. The steps of fibrin formation rapidly follow and in a short period the air vesicles are filled with masses of coagulated fibrin. Exudates of this sort are known as inflammatory exudates in contradistinction to the fluids which pass into the tissue in œdema. The inflammatory exudate is richer in leucocytes and contains fibrin-forming substances which produce deposits of fibrin. Œdema fluid does not contain fibrin-forming elements, but salts, soluble albumins and a very few leucocytes. The first injury to the vessels in pneumonia leads to the passage through the vessel walls of œdema fluid. As the injury to the vessel walls becomes greater, the fibrin-forming elements of the blood plasma pass out and when the injury is sufficient hemorrhage results. The exciting factor of the inflammatory process in fibrinous pneumonia is the diplococcus pneumoniæ (Fränkel) the so-called diplococcus lanceolatus (because of its form). The organisms occur in pairs. In the early stages of the disease it may be detected by spreading the exudate from an alveolus upon a slide or cover-slip and staining after Gram's method. The organism appears blue and in fresh cases lies between the fibrin filaments and the cells, or occasionally within the cells (phagocytosis). As the process advances the organisms lose their staining qualities so that in the later stages of the disease they may be no longer demonstrated. In sections stained with Weigert's fibrin stain the

PLATE XII.

Fig.1.

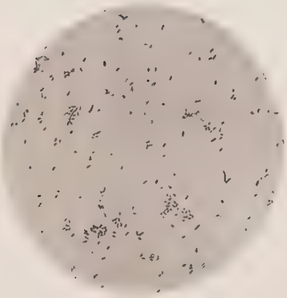


Fig.3.

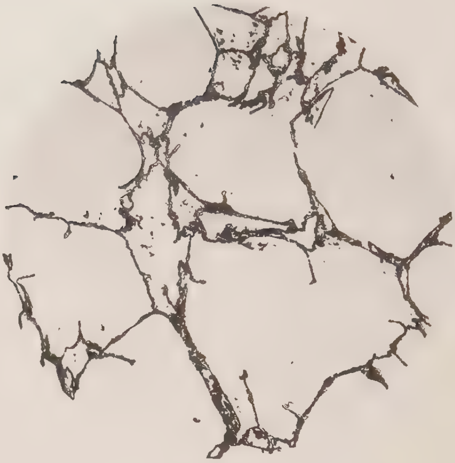


Fig.2.

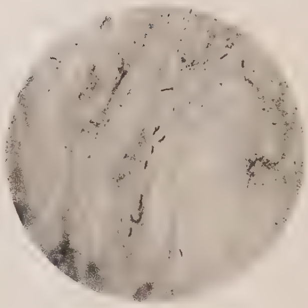


Fig.4.

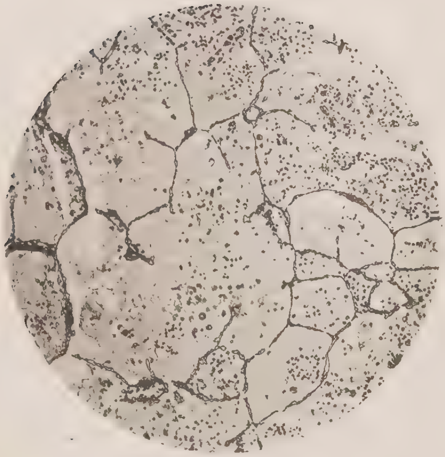


Fig.5.

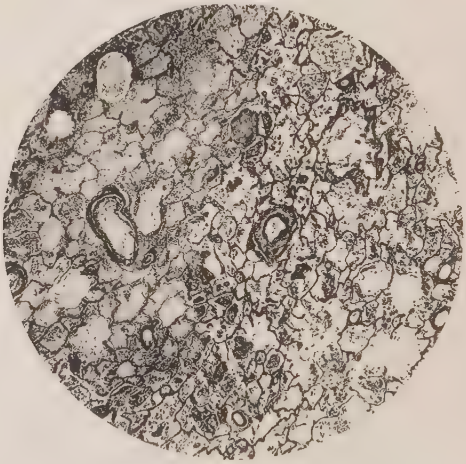


Fig.6.

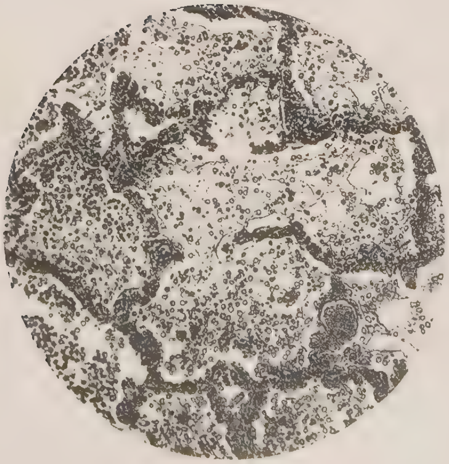
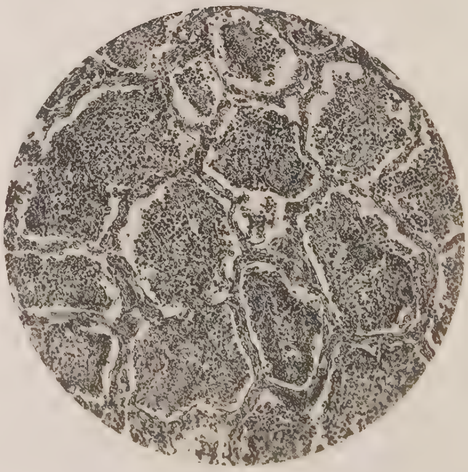


Fig.7.



organisms likewise take on a blue coloration. Occasionally the organisms are arranged in short chains. In the alveolar passages where the organisms are present in the greatest number, the surrounding exudate usually contains a large number of leucocytes and in the alveoli where the fibrinous deposit is thickest, the organisms are generally absent. This rather indicates that the direct irritation of the organisms leads to accumulations of leucocytes and that the action of the toxic substances of the organism produce the exudation of fibrin. The distribution of fibrin and accumulation of leucocytes is not, however, in all cases so typical.

6. Chronic Pneumonia.

In rare cases the fibrin masses are not removed from the alveoli, but remain for a period of time and produce a condition known as chronic pneumonia (carnification of the lung). The masses of fibrin and dead cells bear the same relation to the pulmonary tissue as the necrotic tissue of infarcts bears to the adjacent tissue of the lung or kidney. The process may be most aptly compared to the relations existing between a thrombus mass and the wall of the containing vessel. Exactly as in these cases, the fibrin mass within the alveolus is subjected to the process of organization. When the organization is complete the alveoli are filled with masses of connective tissue, giving the affected lung a tough, leathery appearance and consistence. On section the granular appearance of the lung of pneumonia has disappeared, the tissue offering a smooth, reddish-gray appearance. The markings of the alveoli are obliterated.

F. A., F. M., or embed. Fibrin stain and elastic fiber stain or both combined in one section by staining first with lithium carmine, orcein and Weigert's fibrin method. F., F. M., Weigert's elastic fiber stain and van Gieson. Mallory's connective tissue stain.

Low power discloses the irregular distribution of the fibrin in the alveoli. The various stages of organization are best observed by higher magnification.

DESCRIPTION OF PLATE XII.

FIG. 1. Influenza bacilli. Culture. Oil immersion. Carbol-fuchsin. Very fine short rods.

FIG. 2. Tubercle bacilli in sputum. Oil immersion. Carbol-fuchsin. Slightly curved slender rods.

FIG. 3. Pulmonary emphysema. See p. 153. Middle power. Orcein. Widely dilated air vesicles with flatted epithelium. Extending into the dilated alveoli are short septi. These are old alveolar septi.

FIG. 4. Pulmonary œdema. See p. 135. Middle power. Weigert's elastica stain. The dilated air vesicles and alveolar passages are filled with homogeneous or finely granular material (coagulated albumin) (*magnifier*). Embedded in the coagulated œdema fluid are a number of leucocytes and desquamated epithelial cells (*magnifier*).

FIG. 5. Periphery of a hemorrhagic infarct. See p. 133. Low power. Hæmatoxylin. Weigert's elastica stain. To the left the infarct. To the right air-containing tissue (*magnifier*). Alveoli of infarcted area filled with minute spherical bodies (blood corpuscles). The larger, deeply stained bodies in the alveoli are pigment cells.

FIG. 6. Fibrinous pneumonia. Red hepatization. See p. 137. High power. Hæmatoxylin alizarin. The alveoli are filled with a loose network of fibrin in which are leucocytes (large dark bodies) and red blood corpuscles (somewhat lighter and smaller bodies). The capillaries of the alveolar septi are markedly engorged and widely distended. To the right and beneath a dilated capillary in cross section (*magnifier*).

FIG. 7. Fibrinous pneumonia. Gray hepatization. See p. 138. Middle power. Hæmatoxylin. The alveoli are filled with dense masses of fibrin infiltrated with leucocytes. The capillaries of the alveolar septi are no longer hyperæmic. The fibrin masses are slightly retracted from the alveolar walls (due to hardening).

The alveoli still contain a great amount of fibrin within which may be seen rows of spindle-shaped fibroblasts, usually occupying the crevices. In other alveoli the fibrin is reduced in amount, the number of fibroblasts is greater and newly formed capillaries filled with blood accompany the connective tissue. When the process is advanced a distinct fibrous intercellular substance is visible. The masses of newly formed connective tissue are now seen to penetrate the walls of the alveoli and to be connected by processes of connective tissue in exactly the same manner as the masses of fibrin were connected in the previous stages of the pneumonic process. (Plate XIII., Fig. 1.) From this we see that even the filaments of fibrin which joined the masses have become organized, the fibrin acting as a carrier of the organizing connective tissue through the minute opening in the alveolar septi. The derivation of the newly formed connective tissue is not so readily determined. The principal source seems to be the connective tissue of the respiratory bronchioles. The granulation tissue usually grows from a point in the wall of the bronchiole in the form of a pedunculated polyp which fills out the alveolar passage and the alveoli. Occasionally, however, the newly formed tissue springs from the connective tissue cells of the alveolar septi. The air vesicles are seldom completely filled by the newly formed connective tissue, although the mass is usually adherent to the wall in one or more places. The free spaces between the newly formed tissue and the wall of the vesicle, are frequently lined with epithelium. This is the remains of the alveolar epithelium, but having lost its function it takes on a cubical form and has usually so proliferated as to cover not only the free surface of the air vesicle, but likewise the adjacent surface of the connective tissue. This proliferation usually leads to the formation of cavities lined with cubical epithelium, which present a glandular appearance.

7. Broncho-pneumonia.

In contradistinction to lobar pneumonia, we speak of lobular pneumonia when the process produces disseminated foci, affecting groups of adjacent vesicles usually of the lower lobes. Macroscopically these areas vary in size from pinheads to grains of rice and larger. They are characterized by protrusion above the cut surface, increased density and granular appearance, all of which bear evidence that the alveoli are filled with coagulated masses. When the pulmonary tissue is exposed to pressure, yellowish purulent masses may be expressed from the larger bronchial openings. Scattered among the light gray foci above described may be seen numerous sunken spots of deep red color, from which no air may be expressed. These spots are of greater consistence than the air-containing tissue, but never possess a density equal to the gray foci. Where the number of the focal infections is great, they usually coalesce and in some cases may be so thickly scattered through the pulmonary tissue as to give to one or more lobes the appearance of a true lobar pneumonia.

M. F., F. M., N. S. or Gram or F. A. or embed, fibrin stain.

The examination with low power shows at once in contradistinction to the uniform involvement of lobar pneumonia that only groups of vesicles contain exudate. Within the foci of infection the single air vessels may vary greatly

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Hansemann. Sitzungsber. d. Akad. d. Wissensch. Berlin, 7 Nov., 1895.
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from one another, some being filled with exudate and others practically empty. Very commonly the group of affected alveoli are arranged about a common opening (bronchiolus) or a larger group of affected vesicles may be seen to surround a smaller bronchus, the lumen of which is likewise filled with exudate. (Plate XIII., Fig. 2.) The infection, therefore, affects the terminals of the bronchi and their dependent air vesicles, and consists of a bronchitis of the finer branches of the bronchi and pneumonia of the dependent pulmonary tissue (broncho-pneumonia).

Under high power a more careful examination may be made of the bronchus or bronchiolus, the cylindrical epithelium of which is either found in place or lies detached in the lumen (Plate XIII., Fig. 2) with the exudate. This consists for the greater part of mucus containing a large number of leucocytes. Within the air vesicles the exudate likewise contains a large number of leucocytes, as well as desquamated alveolar epithelium, from which they may be differentiated by their characteristic appearance. The epithelium of the wall of the vesicle when it still remains attached appears swollen and protrudes into the alveolar space. In post-diphtheritic broncho-pneumonia, large masses of protoplasm containing multiple nuclei are found upon the vesicular wall, or lying detached in the lumen. Where the number of leucocytes is small and the amount of desquamated epithelium great, we speak of a catarrhal pneumonia. The amount of fibrin in the affected areas is variable. In some cases only the merest trace of fibrin, or none may be demonstrated; in others the amount of fibrin is equal to that found in true lobar pneumonia. Any relation between the amount of fibrin in the exudate and the number and nature of the exciting organisms, is not demonstrable. At times the process extends from the smaller bronchi directly into the adjacent pulmonary tissue, in which case the walls of the bronchi and bronchioli are infiltrated with leucocytes (peri-bronchitis). Where the irritation is excessive the process may take on a hemorrhagic character, in which case the exudate contains large numbers of red blood corpuscles. All of the alveoli possess a characteristic not found in fibrinous pneumonia. If they be compared with the air-containing alveoli of the unaffected portions of the lung, they will be found to be remarkably reduced in size and in some cases almost completely collapsed. A similar picture is afforded by examination of the red depressed areas noted macroscopically. The condition is plainly produced by occlusion of the air supply, thus allowing the vesicles to collapse (atalectasis). These areas of atalectasis result from occlusion of the supplying bronchus by the inflammatory process, which shuts off the supply of air. As the air remaining in the vesicles is exhausted by the blood the walls of the alveoli collapse, producing the appearance noted. Since the tissue is as vascular as before, and the collapse reduces the volume, it takes on a relatively deeper red appearance than the surrounding air-containing parenchyma.

Broncho-pneumonia is produced by the entrance of organisms into the finer branches of the bronchial tree from which they penetrate into the surrounding atalectatic tissue. It is a common sequel to infections of the upper air passages, as ordinary catarrh, diphtheria, measles, smallpox and scarlet fever. The organisms may be readily detected in sections stained with Gram or Weigert's method. Those most commonly found are diphtheria bacilli, streptococci and pneumococci.

Reference:

Dürck. Studien über die Ätiologie und Histologie der Pneumonie im Kindesalter und der Pneumonie im allgemeinen. Deutsch Archiv f. klin. Med., Bd. 58, 1897.

8. Purulent Broncho-pneumonia.

(*Aspiration Pneumonia.*)

In persistent vomiting, in destructive disease of the upper air passages, or alimentary tract, in new-born which have commenced respiration within the maternal genital tract, irritating or infectious materials are commonly aspirated with the air. The resulting pneumonia is disseminated and localized through the pulmonary tissue, preferably the lower lobes, in exactly the same manner as in broncho-pneumonia, the chief macroscopic difference being that the foci are more yellow in appearance, resembling more closely abscesses, and are surrounded by areas of œdema. A sharp boundary between ordinary broncho-pneumonia and purulent broncho-pneumonia cannot be drawn, as the first is not uncommonly followed by the second.

Cook or F., F. M., N. S., methylene-blue, elastic fiber or Gram.

Under the microscope we find the alveoli filled with polymorphonuclear leucocytes presenting much the appearance of broncho-pneumonia. A distinctive feature is the complete absence of fibrin within the alveoli. In tissue treated with boiling water, or hardened in formaline the leucocytes appear embedded in a finely granular mass which fills the alveoli (œdema). (Plate XIII., Fig. 3.) In advanced cases the entire pulmonary structure within the affected area may be infiltrated with pus cells to such an extent that the markings of the alveoli are obliterated. In many places the tissue is necrosed. The principal characteristic of lobar and broncho-pneumonia is the fibrinous exudate. This is entirely wanting from purulent pneumonia. The process leads to destruction of the pulmonary structure and general purulent infiltration of the tissue. The resulting abscess formation may be compared to septic infarction of the lung except that the infection does not occur through the medium of the circulation. Search must be made for the infecting material within the bronchi. It consists of masses of bacteria with particles of food (stomach contents, vegetable and muscle fibers). Such findings serve to establish the diagnosis of aspiration pneumonia.

Gangrene of the lung is a name applied to the infection and destruction of large portions of pulmonary tissue, followed by softening and putrefaction with the production of disgusting odors. Preparations stained with Gram show that the tissue is infiltrated with different varieties of bacteria and numberless pus cells. The tissue structure is entirely obliterated. Even the elastic fibers of the lung, which of all the constituents of the pulmonary structure are the most resistant, are broken up and liberated and may be detected in the purulent expectoration of the patient. Their detection is best accomplished by cooking the expectorated material with a strong solution of potassium hydrate. The precipitate which settles at the bottom will now contain the elastic fibers, which, if present, may readily be seen, as the pus corpuscles have been destroyed by the action of the caustic potash. The elastic fibers appear as distinct, glistening, wave-like fibers.

The investigation of the lungs of the new-born requires special precautions, for, in these cases, the entire pulmonary tissue may be filled with foreign material without any perceptible microscopic changes. When the trachea and bronchi are carefully opened they are occasionally filled with amniotic fluid, mixed with a certain amount of meconium. This mixture, when examined under the microscope, is found to contain a large number of spindle-shaped plates, the greater number without nuclei. These are the desquamated superficial epithelial cells of the embryo. Mixed with these are a large number of fine hairs likewise from the surface of the embryo, which may be differentiated from filaments of vegetable material by the characteristic pointing of one end; lastly, a certain number of yellow or grayish ovoid bodies, in appearance not unlike the eggs of certain parasites. These are the so-called meconium bodies.

The *fresh examination* may be made by mixing a small portion of the bronchial contents or of the expressed fluid of the lung with salt solution. Where the inspired amniotic fluid reaches the sub-pleural alveoli these may be recognized beneath the pleura by their transparent, yellowish, vesicular appearance and must be differentiated from alveoli distended with air. If the pale areas of injected alveoli be removed and examined, either by teasing or making a mashed preparation, they will be found to contain a fluid similar to that found in the bronchi.

Formaline, F. M., N. S., or Gram.

Under low power the alveoli in which the aspiration has occurred may be recognized from the remainder of the tissue by their increased size and the presence within them of pale, finely striated masses. Beside the inspired desquamated epithelium the fine hairs and meconium bodies are usually present. The hornified cells stain blue with Gram's method. The aspiration of vomited milk in sucking infants may likewise be detected by the fresh examination. The fluid from the lung in these cases contains a large number of free, highly refractive fat droplets of all sizes. These are the normal fat content of the milk. The only thing with which they could be confused would be the fine myelin droplets which are derived from the bronchial epithelium is cases where the cadaver is not fresh.

9. Tuberculosis of the Lungs.

(*Phthisis Pulmonum, Pulmonary Consumption.*)

The alterations of the pulmonary structure included under this heading present wide anatomical differences, with but one factor in common, the specific organism, the tubercle bacillus of Koch. The name tuberculosis was given to the disease because of minute characteristic grayish nodules scattered through the tissue. These nodules are usually from one-half to the size of a millet seed and show a tendency to degenerate from the center toward the periphery. The degenerated portion takes on a yellowish coloration and the material formed is known as caseous material. (See Liver Tuberculosis.) Beside miliary tuberculosis other forms of tuberculous infection of the lungs occur, among them a tuberculous broncho-pneumonia which differs from ordinary broncho-pneumonia by the necrosed tissue breaking down into the typical caseous material of tuberculosis, and by the presence of the specific organism, the tubercle bacillus. The term tuberculosis does not, therefore, include all the anatomical lesions produced by the tubercle bacillus, but is used more specifically to indicate the lesions in which larger or smaller tubercles are formed. On the other hand the term phthisis pulmonum, indicating destruction of the pulmonary tissue, cannot be confined to the destructive lesions produced by the tubercle bacillus, as similar losses of substance result from the ravages of other organisms. The tubercle bacillus is, however, by all means the most common destroyer of the pulmonary structure. Two principal forms of the process may be differentiated:

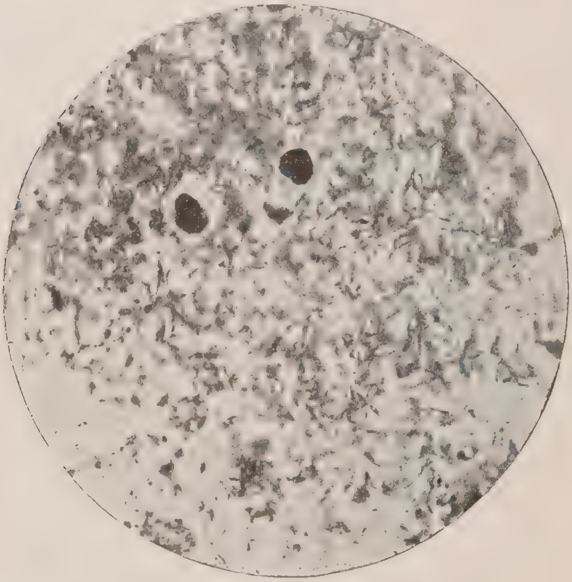
1. That form in which the action of the organism leads to the formation of tuberculous granulation tissue, which is derived from the connective tissue stroma of the lungs or walls of the air passages. This is analogous to ordinary granulation tissue, from which it differs in the tendency of the new tissue to form nodules of definite structure (true tubercle) by the presence of characteristic giant cells, the poor development or absence of blood vessels within the new-formed tissue, and the tendency to hyaline necrosis—the so-called process

of caseation. The various forms of tubercle found in the lung are, in all respects, similar to those described as occurring in the liver.

2. Where the bacillus produces inflammatory exudation. The exudate fills the cavities of the pulmonary tissue and consists of fibrin mixed with different forms of cells and, under the influence of the tubercle bacillus, takes on the typical hyaline necrosis, *i. e.*, caries. It is impossible to describe all of the various combinations which these two principal forms are capable of producing. Only the principal typical forms will be here considered.

(*a*) **Miliary Tuberculosis.**—If tubercle bacilli are disseminated from a tuberculous focus either in the intima of the ductus thoracicus (Fig. 18), the pulmonary veins or the aorta, or from a tuberculous lesion in the endocardium, they are carried with the blood stream to the various organs of the body, where they lead to the formation of minute gray nodules in the connective tissue. In the lung the gray tubercles stand out in sharp contrast to the reddened pulmonary tissue. Where large numbers of bacilli enter the circulation the number of the tubercles in the tissue may be enormous, and in some cases the cut surface is literally peppered with them.

Fig. 18.



Tubercle bacilli in a thrombus in the ductus thoracicus. (Carbol fuchsin. Oil immersion.)

F., F. M., N. S. or F. A. F. M. The student is strongly advised to stain all tuberculous material for elastic fibers, fibrin and the tubercle bacillus. The first three of these steps may be combined in one by staining the section with lithium carmine, orcein and Weigert's fibrin method.

By moving the section under an objective of low magnification, the tissue appears filled with small, clear spaces surrounded by deeply stained borders. Within the clear spaces small, deeply stained bodies are seen, which, upon higher magnification, prove to be giant cells. The deeply stained border of cells is now seen to consist of epitheloidal elements (see Liver Tuberculosis), surrounded by a zone of round-celled infiltration. Here and there a tubercle with caseated center appears. The entire absence of vessels within the tubercles may be especially well seen because of the engorgement of the surrounding capillaries, which end abruptly at the periphery of the tubercles. The adjacent alveoli are concentrically compressed. If the section be stained with orcein or Weigert's elastica stain, the elastic fibers may be traced into the tubercle. (Plate XIII., Fig. 4.) As the tubercle increases in size, the elastic structure is broken up. The fibers themselves, however, show an unusual resistance to the action of the organism, and isolated fragments may be found in the necrotic center of the tubercle. About the periphery the newly formed

Reference:

Benda. Miliartuberkulose, Ergebnisse von Lubarsch-Ostertag, V., 1898.

PLATE XIII.

Fig. 1.

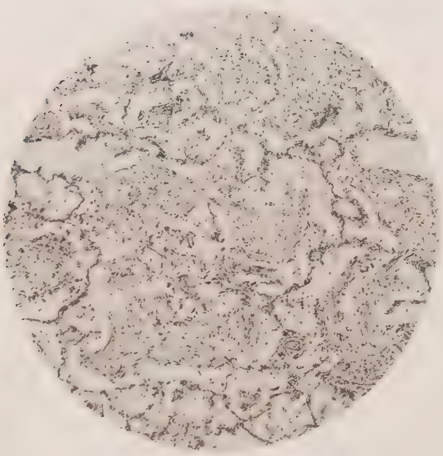


Fig. 2.

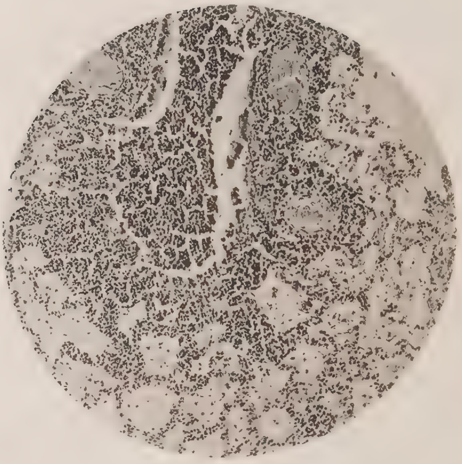


Fig. 3.

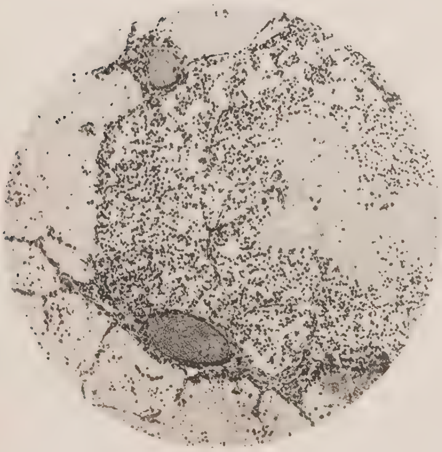


Fig. 4.

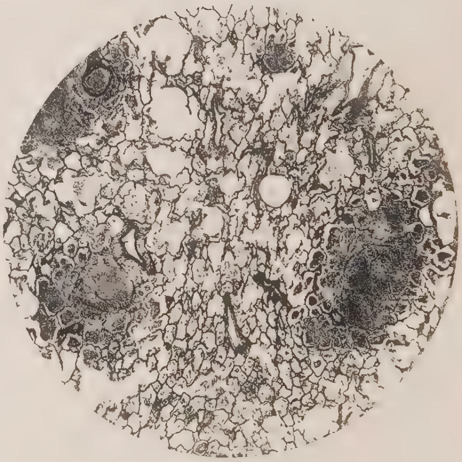


Fig. 5.

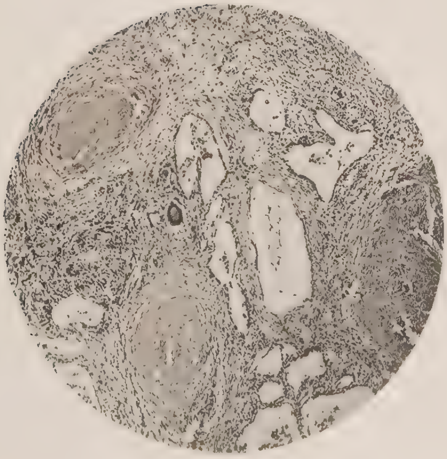
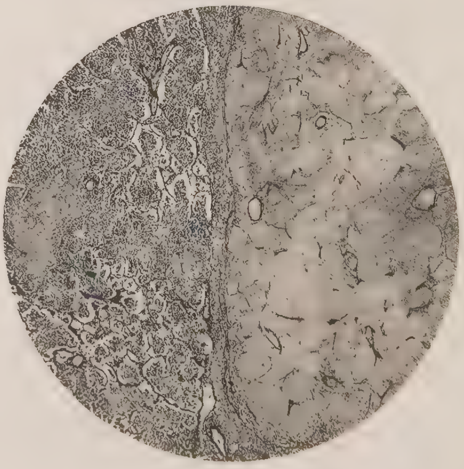


Fig. 6.



connective tissue projects into the openings of the alveoli. If the preparation be stained with Weigert's fibrin method, the necrotic tissue is found to contain a rich fibrin network, showing that the process of necrosis is associated with an exudation of fibrin-forming fluid. In fresh miliary tubercles the adjacent alveoli are free. Where the tubercle has existed for a longer period, the adjacent alveoli frequently contain a fibrinous cellular exudate, which, as the process advances, becomes organized with tuberculous granulation tissue derived from the adjacent alveolar septi, and may entirely fill the lumen of the air vesicle. As the tubercle increases in size and the caseation becomes more extensive, the point of origin of the tuberculous process may be lost. In these cases the process presents an appearance very similar to that found in

(*b*) **Caseous Bronchitis.**—In lungs where tuberculous lesions are present in the upper lobes particles of caseous material are frequently carried with the air current into the lower portions of the organ. The bacilli usually find lodgment at the junction of the bronchi with the respiratory bronchioles and produce a caseous bronchitis and bronchiolitis. The lesions consist of groups of caseous foci, which on superficial inspection might readily pass for large miliary tubercles. Careful examination will discover in the center of many of the caseated areas, a minute opening, which we shall see later is the respiratory bronchiole. Many of the foci contain black coal dust which is deposited in the walls of the bronchi. The distribution of the groups of affected vesicles, follows the terminations of the bronchial tree.

M. F., F. M., N. S.

The affected areas appear under low magnification as small, colorless patches in the nearly normal lung structure. Many appear round, others oval, while some are of irregular shape, having a main trunk and branches springing from the sides, and occasionally with alveolar pouch-like protrusions. In

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DESCRIPTION OF PLATE XIII.

Fig. 1. Chronic pneumonia. (Carnification of the lung.) See p. 139. Middle power. Hæmatoxylin. The alveoli are filled with masses of spindle cell connective tissue. Many of these masses communicate through the stomata in the alveolar walls and are united by slender bands of connective tissue (slightly above center of figure).

Fig. 2. Broncho-pneumonia. See p. 140. Middle power. Hæmatoxylin. From above extending downward is a small bronchus (bronchiolus) lined with typical cylindrical epithelium which is to some extent desquamated. The lumen of the bronchus and the adjacent alveoli are filled with exudate. The alveoli at the periphery contain desquamated alveolar epithelium (*magnifier*).

Fig. 3. Purulent, so-called aspiration pneumonia. See p. 142. Middle power. Hæmatoxylin. The dilated alveoli are filled with coagulated œdema fluid (boiled preparation). The alveoli near the center and to the right contain closely arranged leucocytes. These are so closely packed at the center as to obscure the interalveolar septi. Above and below hyperæmic pulmonary vessels (*magnifier*).

Fig. 4. Miliary tuberculosis of the lung. See p. 144. Low power. Alum carmine. Elastic fiber stain. To the left above and below and to the right three tubercles within which the elastic structure of the lung is well preserved (*magnifier*). The alveoli included within the tubercle are filled with exudate and granulation tissue.

Fig. 5. Chronic miliary tuberculosis of the lung. See p. 147. Middle power. Hæmatoxylin. Above and below to the left hyaline tubercles. Between these a large giant cell with peripheral nuclei. The alveolar septi in the center greatly thickened. The markedly contracted alveoli lined with cubical epithelium (*magnifier*).

Fig. 6. Caseous pneumonia. See p. 146. Low power. Alum carmine. Elastic fiber stain. At the center a pulmonary septum. To the right total caseation of the pulmonary tissue. The position of the interalveolar septi and blood vessels indicated by the remaining elastic tissue structure. To the left of the septum tubercular exudation. At the extreme left beginning caseation.

the larger foci openings with convoluted lining may be seen. The periphery of the caseous masses is bounded by a marked accumulation of epitheloidal and round cells. The fresher the process the more distinctly may the structure of the central bronchioles be determined. As the process advances the adjacent alveoli become filled with cellular and fibrinous exudate followed by caseation. In like manner the process can involve the larger bronchi and the adjacent tissue. Where the caseation has spread beyond the bronchi, the anatomical relations may be readily determined by staining with orcein (or Weigert's elastica stain), when the elastic structure of the bronchus, although in places partly broken up, may be distinctly seen within the area of caseation.

(*c*) **Caseous Pneumonia.**—Where large masses of material containing the tubercle bacilli find lodgment in a terminal bronchus, dependent upon the virulence of the organism, there may develop a lobar or lobular pneumonia, which in its early stages is in all respects similar to true fibrinous pneumonia, except that the consistence of the tissue is somewhat greater, the appearance of the cut surface smoother, more gelatinous, and light gray in color. The color rapidly changes to a yellowish tone and the entire exudate is finally transformed into caseous material.

M. F., F. M., Weigert's fibrin-stain.

Inspection of the section with low power shows at once the great amount of fibrin in the alveoli of the affected portions. When the pulmonary structure is intact, the masses of fibrin in the alveolar spaces are found connected by bands and communicating filaments. This appearance of the structure holds good only for the periphery of the affected lobe. At the center the fibrin is replaced by a poorly stained, slightly granular, homogeneous mass of caseated material. Within the affected area the walls of the alveoli, bronchi and blood vessels are practically obliterated. Here the specific elastica stains are of great service and reveal the elastic structure of the alveoli, bronchi and blood vessels with great clearness. (Plate XIII., Fig. 6.) The elastic structure of the lung resists the necrosing action of the soluble poisons of the organism, but, with the other tissue, is ultimately involved in the general caseation. The cells of that portion of the tissue which lie just at the edge of complete necrosis and within the zone of exudation show evidence of the beginning necrosis of the tissue and the hyaline transformation of the exudate. In this region the nuclei of the cells are distorted and reveal the various forms of karyorhexis or final disintegration with the liberation of drops of chromatin. It will be seen from the above description that the process in these cases is too acute to permit of any extensive formation of tuberculous granulation tissue and that the action of the bacillus produces a fibrinous exudate which is rapidly transformed into caseous material. In many cases, however, the exudative character of the process is not so well defined. The zone which lies between the necrotic center and the periphery of the exudative changes shows marked proliferation of the connective tissue of the septi with the development of interalveolar masses of granulation tissue. The more widely adjacent alveoli are usually filled with œdema fluid, beside a moderate amount of fibrin, leucocytes, and large cells of unusual form. (Plate XIV., Fig. 1.) The protoplasm of these cells is pale, the nuclei deeply stained. Some authors consider them a special form of large migrating cell, others look upon them as altered desquamated, alveolar epithelium. An examination of the alveoli usually discloses in a certain number the presence of an unbroken layer of alveolar epithelium. This certainly indicates that the epithelium of the alveoli is not desquamated *in toto*. The tubercle bacillus may be demonstrated in the tuberculous areas by the usual methods. A form of caseous pneumonia which occurs in children as the result of erosion of a bronchus by a tuberculous lymph node is especially suitable for the demonstration of the organism. In these cases they are usually found in large number in the alveoli adjacent to the caseated areas or in the caseated material. To what extent the tubercle bacillus alone is capable of producing exudation has not been determined. In a certain number of cases of caseous pneumonia it is highly probable that there is a coincident infection by the diplococcus or other organisms.

(*d*) **Chronic Tuberculosis.**—The ordinary form of chronic tuberculosis of the lung begins as an infection of the peribronchial connective tissue, usually in the apices. The organisms are carried to the lung with the inspired air, produce inflammatory changes in the smaller bronchi and rapidly penetrate through the epithelium into the peribronchial connective tissue. Here they enter the lymph channels and where they find lodgment produce tubercles (lymphangitis tuberculosa peribronchialis). Once established the process progresses slowly (chronic tuberculosis). The tubercles in the early stages of their formation present a more or less irregular stellate appearance, produced by the extension of the process along the adjacent alveolar septi. The typical round or oval form of tubercle is rarely found. The organism does not usually possess great virulence, or the virulence rapidly diminishes. As a result the tuberculous granulation tissue is rapidly changed into dense fibrous connective tissue, which walls off the focus of infection from the healthy tissue. The protective process is occasionally sufficient to encapsulate the caseous mass which may become absorbed or replaced by connective tissue. Many of these chronic tubercles never advance to the stage of caseation, but the connective tissue which forms them takes on hyaline degeneration (fibrous or hyaline tubercle). (Plate XIII., Fig. 5.) The caseation process in the smaller bronchi commonly leads to occlusion and the formation of areas of atalectasis. This, with the gradual thickening and increase in bulk of the interalveolar septi, gradually encroach upon the lumenæ of the air vesicles. A certain amount of interalveolar exudation occurs and may undergo organization, which, with the other factors mentioned, gradually transform the surrounding tissue into a more or less dense or fibrous mass. This portion of the tissue is usually impregnated with coal dust which gives it a black, slaty appearance and increased density. The caseated material may become impregnated with lime salts, in which case it appears as dirty, chalky masses scattered through the dark indurated tissue.

Harden in formaline. When necessary decalcify. F. M. Carmine.

The microscopic appearance of the tissue is exceedingly variable, and is dependent upon the size and distribution of the foci of caseation and the number of fibrous tubercles present in the tissue. (Plate XIII., Fig. 5.) The degenerative processes may be absent, in which case the tissue shows various forms of induration, produced by thickening of the trabeculæ through round-celled infiltration, or proliferation of connective tissue. Some of the alveoli are invaded by interalveolar proliferation not unlike that found in chronic pneumonia. Usually the newly formed connective tissue contains more or less extensive deposits of coal dust and even the giant cells may be found to contain fine granular coal pigment. Within the indurated portions of the tissue certain gland-like structures may be encountered. These are nothing more than collapsed alveoli, the epithelium of which, owing to loss of function, has taken on its original cubical form. (Plate XIII., Fig. 5.) Some observers are inclined

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Orth. Ueber käsige Pneumonie, Virchow, Assistenten Festschrift, Berlin, 1891.

A. Fränkel und Troje. Ueber die Pneumonische Form der Akuten Lungentuberkulose, Berlin, 1893.

Sata. Ueber die Bedentung der Mischinfektion bei der Lungenschwindsucht., III. Suppl. zur Ziegler's Beiträgen, 1899.

to believe that these glandular structures represent an attempt at regeneration on the part of the alveolar epithelium.

A form of chronic tuberculosis similar to chronic tuberculous peribronchitis occasionally develops in the lower portions of the lower lobes of lungs which are infected at the apices. The infection occurs in the usual way through the walls of the bronchi and bronchioli and leads to the formation of tubercles in the peribronchial connective tissue. When the tubercles coalesce an appearance is produced which is exactly like the so-called bronchiolitis caseosa. The portion of lung thus affected contains more or less well-defined foci of caseation, within which fine openings may be detected. A characteristic difference, however, is the involvement of the wall of the bronchus in bronchiolitis caseosa. In peribronchitis the caseated area surrounds but does not include the bronchus. Naturally so fine a distinction cannot long exist, as the wall of the bronchus is invariably involved where the process is advanced. In bronchiolitis caseosa the process is usually chronic and associated with the production of fibrous tubercles and the development of fibrous connective tissue which undergoes hyaline transformation. In advanced cases the entire lower lobe of the lung may be thickly sprinkled with indurated nodes containing coal pigment which gives them a black appearance around which fine dots of yellowish caseation may occasionally be detected. The tuberculosis frequently surrounds and isolates areas of tissue which then take on a characteristic yellow ochre coloration. These portions never show the characteristic induration of the infected tissue. When examined under the microscope the yellowish color is found to be due to extensive fat formation in the desquamated alveolar epithelium (see Chronic Œdema.

(*c*) **Tubercular Cavities or Caverns.**—When the tuberculous process invades the pulmonary structure in portions of the lung where the bronchus is still open, especially in the upper lobes, the caseous material is gradually carried away and expectorated. The constant spread of the disease and continued removal of caseous material leads to the formation of cavities within the pulmonary tissue. The walls of these cavities are lined with a thick, gray, slimy membrane, and are marked by ridges of tissue which represent the course of the larger blood vessels, which withstand for a longer period the advance of the disease. Within the wall of the cavity the specific organism encroaches gradually but steadily upon the surrounding tissue and finally carries all before it.

M. F., F. M., N. S.

The wall of a tubercular cavity may be divided into three zones. First, the surface of the membrane, which consists of a thick layer of poorly stained caseous material; second, a zone of granulation tissue; third, the more or less preserved pulmonary tissue which shows the various forms of chronic tuberculosis. In the zone of granulation tissue the activity of the bacilli commonly results in exudation into the alveolar spaces. In some cases this may be sufficient to produce a well-developed caseous pneumonia. The more chronic the process the more extensive is the development of a connective tissue capsule which more or less successfully walls off the tuberculous cavity. (Plate XIV., Fig. 3.) From the superficial layer of caseation particles of caseated material are continually being detached. By the efforts of expectoration these are carried into the bronchi and become mixed with the expectoration of the patient. As this caseated material almost invariably contains tubercle bacilli, the organisms may be detected in the sputum of the patient.

REFERENCE:

F. V. Birch-Hirschfeld. Ueber den Sitz und die Entwickelung der primaren Lungentuberkulose. Deutsches Arch. f. klin. Medizin, Bd. 64, 1899.

In order to detect the bacilli, the sputum is best spread out on a glass plate over a black background, or in a dark-colored dish, and the small, caseous particles are picked out with a needle or small stick. Such particles are mashed between two cover-slips or spread out on a slide, allowed to thoroughly dry, passed three times through the flame, and stained with the specific stain. The bacilli appear as fine rods slightly bent, often in pairs crossed over each other or forming an angle and placed together at the ends. (Plate XII., Fig. 2.) Occasionally a bead-like appearance of the bodies of the organism may be noted.

In many cases the wall of the tuberculous cavern may show an almost complete absence of the necrotic zone and in its place a dense accumulation of polymorphonuclear leucocytes. These pus cells penetrate into the zone of granulation tissue and obscure the typical picture. In such cases, beside the tubercle bacilli, pyogenic organisms will be found in the tissue and within the cavity.

Plate XIV., Fig. 3, is a photograph of the wall of a cavern, in which are seen two blood vessels surrounded by a projection of as yet unnecrosed tissue. These vessels show characteristic changes which consist of proliferation of the connective tissue of the vessel walls (so-called endarteritis obliterans). We see from this that the vessel walls are not immune to the action of the bacilli, although they resist their action for a greater period of time than the surrounding tissue. In the photograph it will be seen that the proliferation of connective tissue is greater in that portion of the vessel which is toward the advancing infection. As the process spreads the proliferation of the connective tissue within the vessels gradually obliterates the lumenæ. For this reason, although the vessel ultimately succumbs to the general destruction, its lumen is already closed, which accounts for the relatively rare occurrence of fatal hemorrhage from the walls of tuberculous cavities.

10. Actinomycosis and Aspergillum Mycosis of the Lung.

The observations of many observers within the last few years indicate that infection of the pulmonary tissue by certain forms of fungi, especially the ray fungus (actinomycosis) and various forms of the aspergillum organism lead to widespread destruction of the pulmonary tissue. The actinomyces organism is usually conveyed to animals and man through the medium of cereals. The infection generally begins in the mouth or pharynx, especially in the neighborhood of carious teeth. Once established secondary infection in the intestinal tract is a frequent sequel and the organism has been found in the œsophagus and vermiform appendix. Primary infection of the mamma, of the skin and lungs have been observed. Where the infection spreads into the surrounding connective tissue it usually leads to the production of widespread abscesses. The pus of actinomycosis is characterized by the presence of the actinomyces organism. This appears in colonies of typical radiate appearance. If pus from an actinomycotic focus is placed in and allowed to flow upon the sides of a test-tube, minute gray-white or yellowish masses will adhere to the glass. When these are examined they are found to be colonies of organisms. To accomplish this they should be placed upon a slide and pressed out under a cover-glass. Under low power the masses appear as spherical or hemispherical bodies of transparent appearance. Under high power the spherical mass is resolved into a radiating structure of club- or flask-shaped elements concentrically arranged. (See Plate XXIII., Fig. 2.) The club-shaped bodies are not always present. The younger the colony the fewer the club-shaped bodies. With the clubbed elements are mixed a certain number of filaments which likewise extend out toward the periphery in a radiating arrangement. As they develop and the colony progresses these fine threads become covered with a hyaline-like material which gives them the clubbed appearance. The development of these clubbed ends is associated with the appearance of the yellow and greenish coloration of the colonies. When the colonies perish they frequently become calcified. Harden in formaline. F. M. The organism is best stained in the tissue by Gram's method or Weigert's fibrin method, preceded by lithium or picro-carmine. The thread-like portion of the fungus is stained dark blue, the clubbed ends reddish or yellow. When stained with hæmatoxylin and Sudan III., the organism is red, the tissue blue. Van Gieson and hæmatoxylin give the same results. In material which is hardened with osmic acid an excessive amount of fat is found in the adjacent tissue. In the hardened section the colonies of the organism are found to be surrounded by accumulations of polymorphonuclear leucocytes which also penetrate into the colonies. (Plate XXIII., Fig. 1.) In the immediate neighborhood of the organism the leucocytes show nuclear degeneration. This accumulation of pus about the organism separates it from the masses of granulation tissue which it produces. The granulation tissue usually contains giant cells and shows advanced fatty degeneration. In old cases the adjacent connective tissue becomes fibrous.

The pulmonary tissue does not, as a rule, afford a suitable substratum for the spores of the ordinary moulds. Where infection does occur the spores are carried into the lung with the inspired air and find permanent lodgment where previous injury of the tissue has produced a locus minoris resistentiæ. Where the spores find lodgment and develop into the mature fungus, this penetrates into the tissue and produces extensive necrosis, followed by the formation of extensive caverns. The starting point of the cavern is invariably a bronchus. Occasionally the elements of the fungus may be detected in the expectoration of the patient, and consists of threads and conidia, or fragments of mycelium.

The tissue should be hardened in formaline or formaline alcohol. Embed in celloidin, stain with van Gieson, methylene-blue or Weigert's fibrin method. Elastic tissue stain. The organism is exceedingly variable in its staining reaction, occasionally being demonstrated by the nuclear, again by the protoplasm, stain. Occasionally it cannot be stained at all.

11. Syphilis of the Lung.

(*Pneumonia Alba.*)

Alterations of the pulmonary structure through syphilis are rare in the adult; in children they are an occasional occurrence. At the autopsy the lungs of children which present the characteristic alterations of syphilis attract attention by the increased consistence and pale color of the pulmonary tissue. In rare cases characteristic gummata may be found in the pulmonary tissue. These have already been described as occurring in the liver. The changes about to be described are those of pneumonia alba.

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Abée. Drei Fälle von tödlich verlaufener Antinomykose. Ziegler's Beiträge, Bd. XXII., 1897.
Schlegel. Ergebnisse von Lubarsch-Ostertag, V., 1898.
Flexner. Pseudotuberculosis hominis streptothricha. Journal of Experimental Medicine, Vol. III., 1898.

Norris and Larkin. Two cases of Necrotic Broncho-Pneumonia with Streptothrix. Journal of Experimental Medicine, Vol. 5, No. 2.
Saxer. Pneumonomykosis supergillina, Fischer, Jena, 1900.

F., F. M., N. S., elastic fiber stain.

The characteristic microscopic appearance of the tissue is found in the thickened interalveolar septi and in the poor development of the alveoli, which may be so few in number that under a lens of low power only one or two may be seen in an entire field. The alveoli are small and the epithelium is cubical, as in the developing lung of the embryo. The appearance of the tissue suggests that the alveolar development has remained stationary from an early period of interuterine life, and the increased bulk of the lung has been supplied by connective tissue proliferation in the septi. (Plate XIV., Fig. 4.) In other cases the size of the alveoli is about normal with the interalveolar walls somewhat thickened. The alveolar epithelium is often desquamated and the interalveolar spaces contain numbers of polymorphonuclear leucocytes.

Fresh double knife sections treated with potassium hydrate show marked fatty metamorphosis of the alveolar contents. Desquamation of the alveolar epithelium occurs in the lungs of still-born embryos and as the alveolar contents, which are likewise richly mixed with leucocytes take on a marked grade of fatty degeneration, the condition cannot be considered characteristic of syphilis. The thickened trabeculæ of the lung, which show evidence of active proliferation, would be the distinctive feature in the determination of syphilis.

12. Pneumoconiosis.

The constant inspiration of air containing various forms of foreign matter in suspension (coal, stone and brick dust, particles of iron, etc.) produces in the course of years deposits of these substances in the lungs followed by alterations of the tissue, which are known as anthracosis, calcicosis, siderosis, etc.

(*a*) **Anthracosis.**—The substance most frequently conveyed to the lung with the inspired air is coal dust. In spite of the activity of the ciliated epithelium of the trachea, particles of coal dust find their way into the air spaces of the respiratory parenchyma, and are taken up by the epithelial cells lining the air vesicles. (Plate X., Fig. 3.) Through gradual desquamation these coal-laden cells become detached and may often be detected in the morning expectoration of healthy individuals, where they appear as mononuclear elements with large cell bodies, the protoplasm of which is loaded with black angular granules (dust cells). The pigment found in these cells is unaffected even when treated with strong acids (sulphuric acid), which distinguishes it from dark hæmatogenous pigment, which rapidly disintegrates under similar treatment. By this means we may determine that the pigmentation is due to deposits of coal dust. The lungs of individuals who have resided for long periods in industrial centers commonly contain extensive deposits of coal dust. These are usually so extensive as to preclude the possibility of the coal dust being deposited in the alveolar epithelium alone. The distribution of the coal dust may be determined by the examination of sections. F., F. M., carmine and elastic fiber stain.

The microscopic picture naturally varies with the extent of the deposits and under low power the tissue presents a considerable similarity to the conditions found in cyanosis of the lung, with the exception that the pigment in the epithelial cells and connective tissue stroma of the septi is black instead of brown. (Plate X., Fig. 2.) In the portions of the lung where the coal deposit is greatest an occasional alveolus entirely filled with coal pigment is encountered. The connective tissue structure of the septi is increased in amount, the trabeculæ are markedly widened and densely impregnated with coal. In many places

REFERENCE:

Flockemann. Zus. Referat über Lungensyphilis. Centralbl. f. allg. Path., Bd. X., 1899.

the thickening of the septi is so extensive that the alveoli are obliterated. The deposits of coal dust are usually excessive in the neighborhood of the smaller lymph nodes. These may be detected upon the pleural surface as grayish white bodies with intense black borders. The changes to which they are subject will be considered under Anthracosis of the Lymph Nodes.

Chronic inflammatory conditions of the lung predispose to the deposition of coal pigment. For this reason chronic tuberculosis is almost invariably accompanied by anthracosis. Where the coal dust is deposited in the neighborhood of vessels it commonly penetrates into the vessel wall and may occasionally be found even in the intima of the vessel. It occasionally happens that lymph nodes containing large amounts of coal dust lying adjacent to blood vessels involve the vessel in the process of disintegration, and that the contents of the lymph node are emptied into the vessel. When this occurs considerable amounts of coal pigment may be carried into the general circulation, and are deposited in the spleen, kidneys and liver.

(*b*) **Calcicosis. Stonecutter's Lung.**—The so-called stonecutter's lung is distinguished by the presence in the pulmonary tissue of hard, fibrous nodules in appearance not unlike fibrous tubercles. These consist of circumscribed masses of connective tissue containing deposits of stone dust and are the result of irritation of the connective tissue by the silicic acid and calcium salts of the stone dust.

F. Decalcify. F. M. Carmine (van Gieson, elastic fiber stain).

REFERENCE :

J. Arnold. Untersuchungen über Staubinhalation und Staubmetastasen, Leipzig, 1885.

DESCRIPTION OF PLATE XIV.

FIG. 1. Alveolus from the œdematous margin of caseous pneumonia. See p. 146. High power. Hæmatoxylin, elastica stain. The contents of the alveoli consist of leucocytes and large round epithelial elements (so-called exudate cells or desquamated epithelium).

FIG. 2. Stonecutter's lung. (Calcicosis.) See p. 153. Very low power. Hæmatoxylin, elastica stain. Large central fibrous node surrounded by smaller nodes of the same character. The remains of the elastic structure of the lung may be seen in the fibrous structure of the node. Scattered through the tissue are occasional chronic miliary tubercles (*magnifier*).

FIG. 3. Wall of a tuberculous cavity. See p. 148. Very low power. Hæmatoxylin, elastica fiber stain. Above margin of cavity. To the left and right caseation extending into the pulmonary tissue. In the wall of the cavern are four vessels, two lying near the surface in a projection of thickened pulmonary tissue. The more superficial of these vessels show eccentric endarteritis productiva with the greatest thickening toward the surface of the cavity. The second vessel shows more concentric thickening (*magnifier*).

FIG. 4. Congenital syphilis of lung. (Pneumonia alba.) See p. 150. Low power. Hæmatoxylin. The interalveolar septi greatly thickened. Some of the alveoli affected by atalectasis. Others emphysematous. Above the remains of a disintegrated bronchus, the epithelium of which is scattered through the stroma. To the right a blood vessel showing syphilitic endarteritis.

FIG. 5. Lymph vessel carcinoma of the lung. See p. 136. Middle power. Cooked preparation. Hæmatoxylin. To the right a large artery which is surrounded on the left by an extensive crescentric mass. This is composed of closely packed epithelial cells which occupy the greatly dilated perivascular lymph space. The deeply stained nuclei of the carcinomatous cells may be readily discerned. The adjacent pulmonary alveoli are filled with coagulated œdema fluid (*magnifier*).

FIG. 6. Diphtheritic membrane in the pharynx. See p. 159. Low power. Resting upon the tissue is a thick network of fibrin which to the left is supported by a deeply stained basal membrane from which the epithelium has disappeared. To the right the fibrin is intimately connected with and extends into the tissue. Vessels hyperæmic. Superficial structure of the mucous membrane infiltrated with leucocytes (*magnifier*).

PLATE XIV.

Fig. 1.

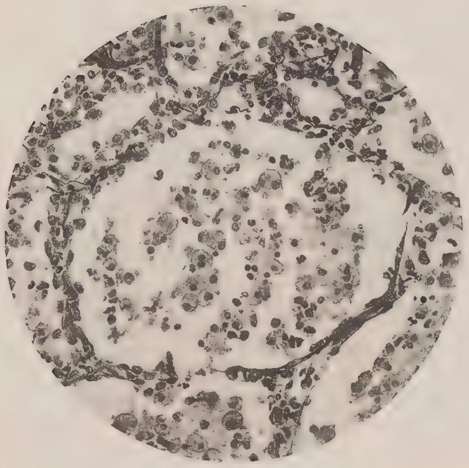


Fig. 2.

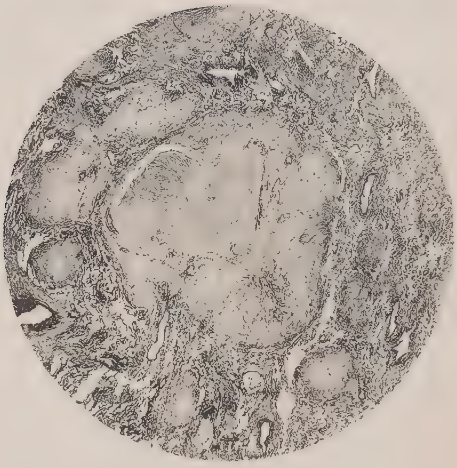


Fig. 3.

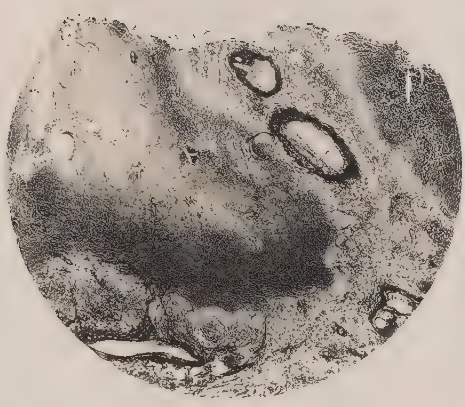


Fig. 4.

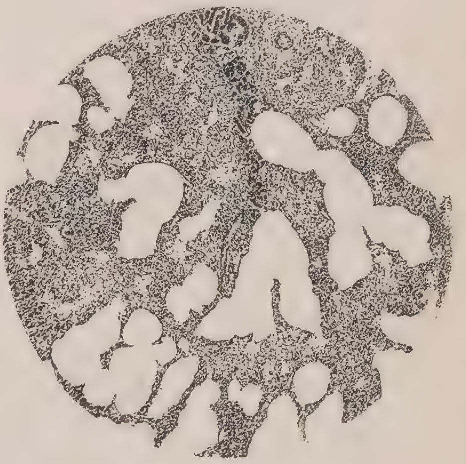


Fig. 5.

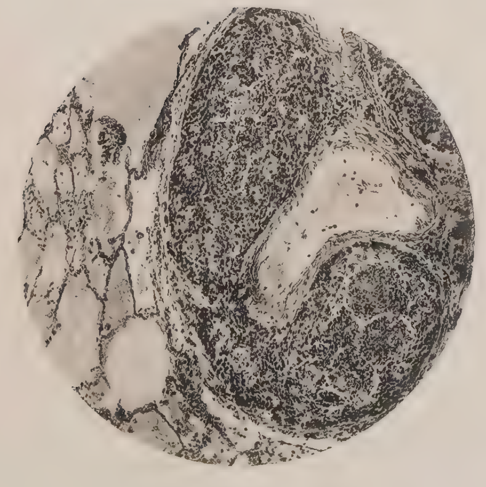
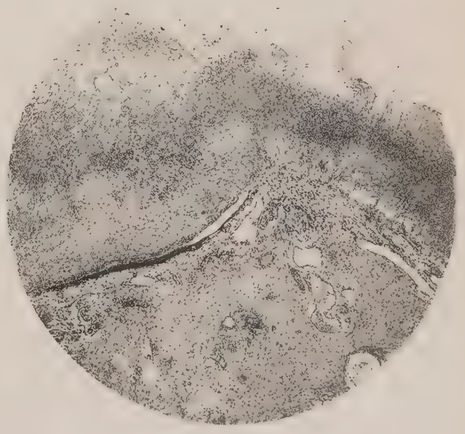


Fig. 6.



Under the microscope the nodules, to a certain degree, still resemble fibrous tubercles. They are made up of fibrous tissue of hyaline appearance, with more or less characteristic concentric lamination. (Plate XIV., Fig. 2.) The cells at the centers of the nodes fail to take the nuclear stain, but their outlines are usually visible, although transformed into hyaline structures. Calcicosis is frequently complicated by anthracosis, for which reason we find more or less extensive deposits of coal dust in and about the nodes. The coal dust deposited about the periphery is usually in the characteristic spindle-shaped deposits, within which nuclei may be occasionally demonstrated. At the center, where the cells are broken up, the pigment is disseminated through the structure. Thus far the structure closely resembles that of fibrous tubercles, with the exception that the characteristic giant cells which should be at or near the periphery are absent. By this means we may differentiate the fibrous nodes from tubercles. Most cases of calcicosis are, however, associated with chronic tuberculosis. In these cases true tubercles with caseation and giant cells may be found coincident with the characteristic connective tissue nodules produced by the action of the silicic acid from the stone dust. Differentiation becomes more difficult where the tubercles are not caseated or do not contain giant cells. This difficulty is likewise increased by the occasional presence of foreign-body giant cells about the periphery of the stone-dust nodules. Although the giant cell is characteristic of tubercle, giant cells of more or less typical appearance may be found in other processes than tuberculosis, most commonly about chronic inflammatory foci of connective tissue.

(*c*) **Siderosis.**—The material deposited in these cases is iron oxide, which gives the lungs a red coloration. The metallic dust is deposited in a manner similar to the coal dust in anthracosis.

13. Emphysema.

In all cases where the pulmonary structure is exposed to excessive and persistent interalveolar pressure, the alveoli become dilated. This dilatation may be so marked that the cut surface of the lung, instead of displaying the barely visible alveoli, may present numerous vesicular openings as large as peas or cherries, situated most often near the pulmonary margins. The very beginning of this process is best seen in lungs affected with focal inflammatory processes, where, owing to the diminution of air space, the vesicles adjacent to the foci develop a compensatory dilatation. The most exaggerated forms of emphysema are found in special cases of chronic bronchitis, asthma and occasionally in the senile, where the dilatation is due to loss of tone in the pulmonary tissue, which, associated with contraction of the bronchi, leads to extensive dilatation, the result of interalveolar pressure at the moment of expiration.

F., celloidin or paraffine. Fasten to slide. N. S. Elastic fiber stain.

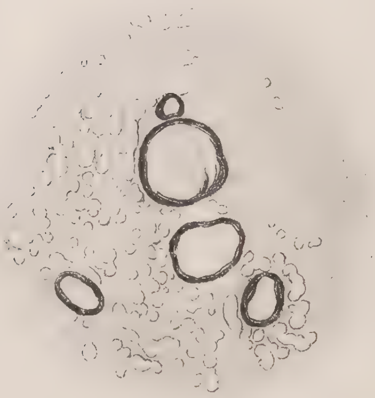
The microscopic picture presented under low power is that of a coarse network of tissue containing more or less irregular openings. Usually short septi extend into the openings. (Plate XII., Fig. 3.) The larger openings are the dilated alveolar passages and the projections of tissue which extend into them are the shortened alveolar septi. Excessive dilatation of the interalveolar spaces is associated with gradual thinning and atrophy of the pulmonary structure. The first evidence of the change is found in the dilatation of the stomata in the alveolar walls. Careful examination of the changes which occur can only be made by staining tissue with an elastic fiber method and injecting the

Reference:

Nölke. Ueber experimentelle Siderosis. Arch. f. experiment. Pathol., Bd. 43, 1900.

vessels. By the first method we may determine that the elastic fibers are affected by the general atrophy and we may with injected material observe the changes which occur in the capillary network.

Fig. 19.



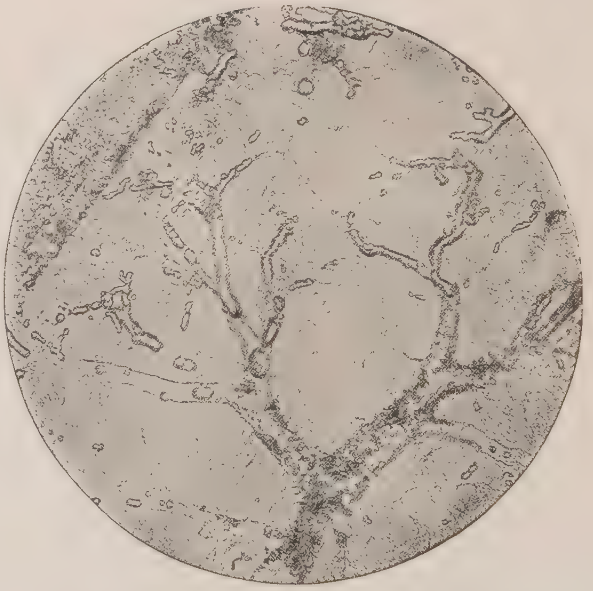
Fat emboli in the lung (fresh shear cutting). The pale branching bodies are fat, the large oval and round bodies with dark contours, air bells; to the right a suggestion of the stroma.

14. **Fat Emboli in the Lung.**

In grave traumata, especially where the tissues have been extensively crushed, and in multiple or extensive fractures of bone, the subcutaneous fat, or the fat of the yellow bone marrow, finds its way into the venous system, and being transported to the lungs lodges in the pulmonary capillaries. The amount of fat which in this way becomes lodged in the pulmonary tissue is occasionally sufficient to prevent respiration. Therefore, in all cases of sudden death following extensive injury, protracted labor, etc., careful examination of the lungs for fat emboli must be made.

Fresh examination suffices. Small pieces of lung tissue removed from the cut surface with the curved shears are examined in salt solution. These are pressed out under the cover-glass without undue pressure, which would cause the fat to leave the capillaries.

Fig. 20.



Fat emboli in the branches of a pulmonary vessel. Fresh preparation treated with potassium hydrate. (Redrawn from Kaiserling, Compend. d. wiss. Photographie.)

Any air bubbles which may have entered the preparation must be carefully removed by pressing upon the cover-glass with a needle. Fat emboli may not be found in the first preparation, but when they are present in any considerable number, a few trials will usually disclose the characteristic appearances which they present. (Fig. 19.) The air bubbles may be readily differentiated from the fat droplets. In the former the bright spot appears on focusing downward, in the latter on focusing upward. The fat is lodged in the pulmonary tissue and consists of pale, shining, irregular masses, with delicate contour, which are usually fusiform, branching, or form a coarse network in the tissue. By the clearing of the tissue, on the addition of potassium hydrate, these become more definite, and are clearly seen to lie within the capillaries. (Fig. 20.) A certain amount of fat naturally passes through the pulmonary network and finds its way into the greater circulation. In these cases it is usually possible to demonstrate a certain number of fat emboli in the kidney where they occlude the loops of capillaries in the glomeruli.

15. Tissue and Cell Emboli in the Lungs.

In a variety of pathological conditions single cells or groups of cells may become detached and be carried into the pulmonary circulation where they lodge in the capillaries. In serious injury to the liver substance, in eclampsia and in different infectious diseases, groups of liver cells have been found plugging the smaller arterioles of the lung. In eclampsia, placental cells and small fragments of the chorionic villi may become detached. These act in the same manner. The giant cells of the bone marrow in a large number of toxic and infectious diseases and following severe burns have likewise been found in the pulmonary capillaries. Beside fat emboli, complete fat cells and fragments of fat tissue or even of bone marrow have been found in the lungs after severe injury to bone. All of these tissue emboli require hardened tissue and cut sections for the determination of their presence. Where the emboli are fresh the character and appearance of the cells which form them may be recognized. Later they break down and lose their characteristics.

VII. THE MUCOUS MEMBRANES.

1. Bronchitis.

Structure.—The histological structure of the bronchi is as follows: The outer portion of the bronchus, which is continuous with the pulmonary tissue, consists of connective tissue structure rich in elastic fibers which here and there include islands of cartilage. Directly within this outer connective tissue structure is an interrupted circular muscular ring which likewise contains a large number of elastic fibers. Within this lies the mucous membrane which consists of a groundwork of connective and elastic tissue and a hyaline basement membrane upon which rests the epithelial lining of the bronchus. This consists of multiple rows of ciliated cylindrical epithelium with a considerable number of goblet cells. Embedded in the wall of the bronchus are a large number of mucous glands. These lie in the connective tissue structure between the muscular and peripheral cartilage-containing coat. The openings of these glands penetrate through the muscular coat to the lumen of the bronchus.

In acute mucous and purulent catarrh the lumen of the bronchus contains a tough, glassy, or yellowish purulent exudate. When this is examined in the fresh state it is found to consist of a more or less homogeneous groundwork in which are embedded a large number of ciliated epithelial and widely distended goblet cells (mucous cells). Occasionally the mass is permeated with hyaline drops and large coarsely granulated cells. A certain number of the latter are leucocytes in an advanced state of fatty degeneration, but a larger number of them contain one or two nuclei and have regular, highly refractive granula. These last are eosinophile cells and in certain cases of asthma form the principal cellular contents of the mucous exudate. In these cases the secretion likewise contains a large number of hexagonal crystals (Charcot-Leyden's crystals).

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When the mucus is spread out upon the slide or cover-glass, the freed granula are scattered through the mucus in rows or bands. If acetic acid is added to the preparation the periphery of the masses of mucus take on a characteristic coarse or fine striation. This does not extend through the mass as the coagulation at the periphery prevents the action of the reagent in the deeper portions of the mucus.

If *cover-glass preparations* are made from the mucous secretion in cases of asthma, and stained with eosin, the eosinophile cells may be recognized by the brilliant red staining of their granula. Counterstained with methylene-blue the cells are found to possess one or two deep blue nuclei. Any bacteria which may be included in the preparation are likewise stained by this method. For purposes of control other cover-glass preparations should be stained with Gram's method. Various organisms have been found to be the cause of the different forms of bronchitis (diplococcus pneumonia, Friedlander's bacillus, etc.). An organism very commonly encountered in these cases is the influenza bacillus. (Plate XII., Fig. 1.) This may be stained with dilute carbol-fuchsin (several minutes) and appears as extremely minute, slender rods, usually in groups. The organism may be stained in the tissue according to Nauwerck by staining over night in dilute carbol-fuchsin solution and decolorizing with 80-per-cent. alcohol.

Material for hardening should be taken from the larger bronchi and should include the entire lumen. F., F. M., or embed. N. S., Elastic fiber stain. (Methylene-blue.)

Under low power the reddening and swelling of the mucous membrane can be traced to the engorgement of the superficial blood vessels. About the blood vessels and directly beneath the basal membrane are large numbers of closely packed cells, the presence of which likewise contributes to the swollen condition of the membrane. Under high power these cells are found to be poly- and mononuclear leucocytes. The infiltration extends to the surface where the cells are accumulated in groups, illustrating the manner in which leucocytes penetrate through the epithelial covering and become part of the exudate in the bronchi. The continuity of the epithelial covering is occasionally broken. This, in some cases, is due to burrowing of the leucocytes beneath the epithelium. Desquamation of the epithelium is a common post-mortem phenomenon which fact must be taken into consideration as a possible factor where the epithelium is detached. The openings of the mucous glands are usually widely distended with material which, in methylene-blue preparations, takes on a deep blue color. The deeply-stained material is mucus and is likewise found within the bronchial lumen, where it forms the greater part of the exudate. The goblet cells may be readily differentiated in these preparations and appear as widely distended cells containing large spheres of deeply-stained mucus. In chronic bronchitis the wall of the bronchus presents numerous transverse ridges. The membrane covering them is finely granular and distinctly reddened.

M. F., F. M. or embed. N. S., van Gieson. Elastic fiber stain.

Material to be investigated should include both cross and longitudinal sections of the bronchi. Sections in the longitudinal axis show the projecting ridges which are often polypoid in form. The entire wall of the bronchus is richly infiltrated with cells. This infiltration is either diffuse or the cells are grouped together in masses. (Plate XVI., Fig. 4.) Under high power the projections

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on the surface of the mucous membrane are found to contain cross sections of muscle bundles. The muscle fibers within these bundles are markedly enlarged. After careful orientation we may determine that they consist of hypertrophic fibers from the circular, muscular coat of the bronchus. These muscle fibers are usually located near the base of the polypoid projections, which are formed by folds of mucous membrane and contain widely distended capillaries. The surface of these projections is usually covered with a single layer of flattened epithelium, in place of the multiple layers of ciliated epithelium which forms the normal lining of the bronchus. The wall of the bronchus to its outermost covering is richly infiltrated with round cells. When the infiltration extends into the adjoining tissue the condition is known as chronic peribronchitis. The development of granulation tissue occasionally follows the round-cell infiltration and after undergoing the usual fibrous changes, transforms the wall of the bronchus into a mass of fibrous connective tissue (fibrous bronchitis and peribronchitis). This change usually affects the smaller bronchi. In other cases the round-celled infiltration becomes more extensive and permeates between the mucous glands which ultimately atrophy and disappear. The round cells and granulation tissue invade the cartilage and split up and destroy the elastic tissue structure. As a result the wall of the bronchus becomes softened and loses its elasticity and when exposed to excessive interbronchial pressure, dilates, producing the condition known as bronchiectasis. For a considerable period of time the remains of the muscular coats and elastic tissue structure of the bronchus may be detected in the walls of the dilated cavities, but later gradually atrophy and disappear. The granulation tissue slowly takes on the usual fibrous change, and the wall of the cavity may be transformed into a distorted fibrous membrane.

2. Tuberculous Trachitis.

Structure.—Rigidity and elasticity are given to the wall of the trachea by a series of horseshoe-shaped cartilaginous structures. The openings of these structures are in the posterior wall where the deficiency is supplied by bundles of smooth muscle fibers. These segments of cartilage surround the trachea and are held together by connective and elastic tissue. Directly within the rings is the submucosa of the tracheal lining, which contains a large number of mucous glands. In the posterior wall the mucous glands extend through the muscle bundles which connect the ends of the cartilage and are sometimes found outside them. A distinct layer of elastic tissue separates the submucosa from the mucosa. The openings of the mucous glands extend through this and the mucosa to the surface of the tracheal lumen. The mucosa contains a large number of capillaries and on the surface is condensed to a definite basal membrane. Upon this membrane rests the layers of ciliated epithelium which, like that of the bronchi, includes a considerable number of goblet cells.

The catarrhal changes in the trachea differ in no way from those affecting the bronchi. Occasionally the excretion of mucus in catarrh of the trachea becomes excessive and in such cases, the surface of the trachea is covered with minute clear drops which mark the openings of the mucous glands.

The tuberculous infections of the trachea are more important. Masses of caseous material which are detached from tuberculous foci in the lungs become mixed with the secretions of the bronchial tubes and are ultimately expectorated by the patient. In their passage from the pulmonary parenchyma to the pharynx small particles of caseated material containing bacilli become mixed with the stagnated secretions of the trachea. The organisms are thus afforded an opportunity for invasion of the tissue, which they accomplish by penetrating the epithelium of the tracheal wall. The path which the caseous material follows in these cases is marked by a distinct trail of minute tuberculous ulcers extending through the larger bronchi and the entire length of the trachea, where they usually appear upon the posterior wall.

M. F., F. M., N. S., or tubercle bacilli stain.

Longitudinal or transverse sections of the tuberculous trachea when examined under low power, are found to contain a large number of deeply stained foci, directly beneath the epithelium. These may or may not show central caseation, but the presence of giant cells within them and their characteristic structure usually discloses their identity. Under high power they are found to be distinct tubercles. In most cases the layer of epithelium directly above the tubercles is unbroken. Where the caseation which begins in the center of the tubercles extends to the surface, the continuity of the epithelial covering is destroyed. As the caseated material is disintegrated the tubercles are rapidly transformed into ulcerating defects in the mucous membrane. These constitute the small ulcers observed with the naked eye. Many of them are located near the openings of the mucous glands. When properly stained the bases of the ulcers usually contain large numbers of tubercle bacilli. Tuberculous infections of the bronchi and larynx progress in a similar manner. A special form of tubercular infection is found in the larynx, especially in the neighborhood of the ary-epiglottic folds. In this form caseation and ulceration are less common, the infection producing an infiltration which leads to thickening of the mucous membrane (infiltrating tuberculosis). Beside the thickening, polypoid proliferations have been observed upon the surface of the membrane. The thickening and proliferation of the tissue in these cases is due to large numbers of deeply embedded tubercles (Plate XVI., Fig. 5) and excessive, diffuse, round-celled infiltration.

3. Pseudo-Membranous Inflammation of the Mucous Membrane.

The term diphtheria has been applied to the inflammatory affections of the mucous membrane which are associated with the formation of pseudo-membranes. These pseudo-membranes may rest somewhat loosely upon the mucous surface, or may be intimately connected with the superficial layers of the underlying tissue. The microscopic examination in the first case shows that the membrane consists of fibrin deposited upon the surface of the membrane. In the second case this deposit of fibrin is associated with necrosis of the superficial layers of the mucous membrane, or in some cases may even consist of a simple necrosis of the tissue without any well-defined exudation. In most cases the two processes are combined, and as the name "diphtheria" is employed among clinicians to designate a characteristic, acute, infectious disease, it would seem desirable to describe these mixed forms of fibrinous exudation and necrosis of the mucous membrane under the heading "pseudo-membranous inflammations of the mucous surfaces," which obviates the necessity of an anatomical distinction.

Pseudo-membranes may develop on the surface of any mucous membrane by the action of organisms or various toxic substances which are capable of causing injury and necrosis of the tissue. According to the action of the irritant, the membrane is either superficial and partakes of the exudative type, or is more adherent, in which case the underlying tissue is necrotic. Although bacteria are commonly the active agents in the production of pseudo-membranes, a typical pseudo-membrane may be produced, either by the action of strong mineral acids or concentrated alkalies, and well-developed pseudo-membranes are found in the intestines of patients suffering from uræmia, being probably the result of irritation by certain toxic substances elaborated in the system of the sufferer. The best example of pseudo-membrane formation due to the action of a bacillus, is found in diphtheria. The infective agent in this case is invariably a bacillus known as the Klebs-Loeffler bacillus, and its action is most commonly localized in the upper air passages (*i. e.*, pharynx and larynx). The action of the bacillus in these cases leads to the formation of a typical grayish white pseudo-membrane. A similar membrane occasionally develops in the course of scarlet fever. In these cases, however, the pseudo-membrane presents more the appearance of crusts and the infection is usually due to a streptococcus. It is well to remember that in true diphtheria other organisms, beside the specific bacillus, enter the tissue and take part in the infection. In fact, in the later stages of the disease, it is possible for the Klebs-Loeffler bacillus to entirely disappear and for other organisms (bacteria and cocci) to remain. This form of double infection is probably the cause of many of the severe cases of diphtheria which are encountered. The reverse condition unquestionably occurs when diphtheria is combined with scarlet fever, in which case the infection is primarily due to the invasion of the tissue by a coccus, with a subsequent infection by the diphtheria bacillus. In both cases the primary destruction of continuity of the tissue opens the way for the entry of organisms which are commonly present in the cavities of the mouth and pharynx. The anatomical relations in the naso-pharynx and trachea cause marked structural differences in the membranes formed in these localities. It is, therefore, well to consider them separately.

The histological structure of the mucous membrane covering the cavity of the mouth and pharynx is, in general, the same. They consist of three layers, a superficial covering of epithelium, supported by the mucosa or tunica propria which possesses papillary projections and the submucosa. The epithelium is of the squamous variety and consists of several layers. The mucosa contains a large number of lymphoid nodes. From these are derived a large number of migrating lymphocytes which normally penetrate through the epithelial covering. The submucosa contains a large number of mucous and albumin-excreting glands. There is no basal membrane supporting the epithelium of the mucosa, as in the trachea and bronchi. That portion of the laryngeal mucous membrane which is continuous with the trachea is covered with ciliated epithelium and possesses a similar structure to that of the trachea. The mucous membrane of the vocal cords is similar to that of the pharynx and supports a covering of squamous epithelium.

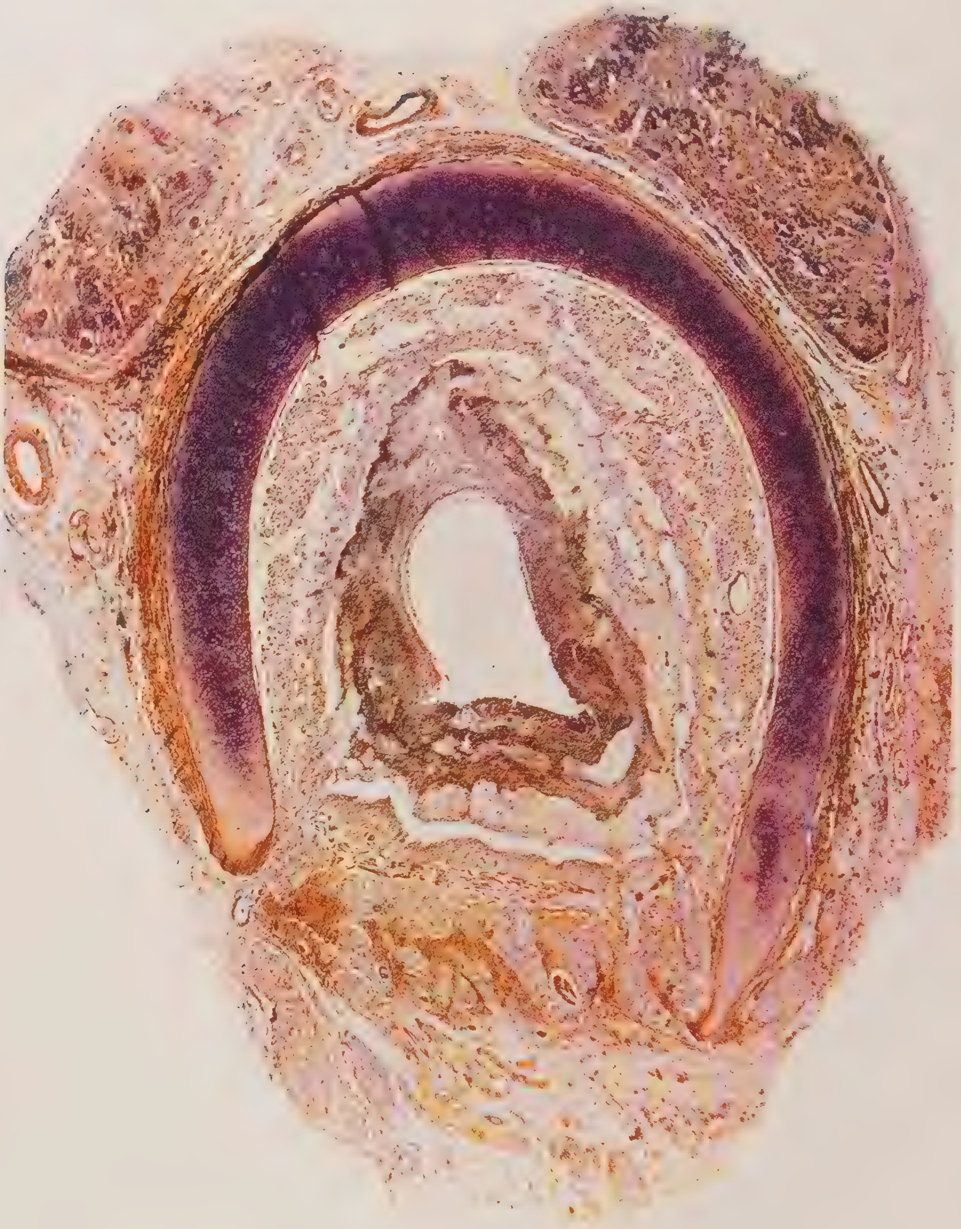
A. **Diphtheria of the Palate.**—The diphtheritic membrane makes its appearance in the naso-pharynx as a thick, grayish yellow deposit. If, either on the living patient or the cadaver, the investigator attempts to remove a portion of this membrane, it will be found that the grayish white mass is tenaciously adherent to the tissue, and that after removal the surface from which it has been stripped has a raw, denuded appearance. In a living patient the removal of the membrane is often associated with vigorous hemorrhage from the denuded surface, indicating that the superficial capillaries have been torn. If a portion of the grayish white mass so removed be *teased in salt solution* and examined under the microscope, it will be found to consist of a network of highly refractive threads and bands intimately interlaced. Examined with higher magnification these threads and clumps are found to possess a finely granular structure, intimately suggestive of the threads and masses found in freshly coagulated fibrin. On the addition of acetic acid the granular appearance of these masses and threads disappears, leaving only the highly refractive homogeneous masses which we have already observed, and which we may now recognize as hyaline in character. These hyaline masses are generally considered to be a degeneration product derived in part from the tissue cells and from the fibrinous deposits. It is probable that the cells which go to form the hyaline masses are in the first place impregnated with the fibrin-forming fluids which exude from the injured capillaries. These cells, losing their vitality, take on the hyaline change, forming the highly refractive bundles and masses described.

The finer structure of the membrane is best observed in preparations which have been subjected to hardening and fixing processes, properly embedded and sectioned perpendicularly through the surface. Sections thus prepared are best stained with picro-carmine, in which the fibrin threads and masses are stained yellow, or with the specific Weigert fibrin method, in which the same structures are stained a deep blue, and their relations to the tissue may be readily determined.

If the deposit be in the form of a minute island of pseudo-membrane, this, when examined under low power, is seen to consist of a deep blue membrane with a wide base which extends down into the mucous membrane and a mushroom-like top which spreads itself out over the adjacent squamous epithelium. The edges of this membrane are now seen to consist of a coarse network of fibrin containing a few polymorphonuclear leucocytes. The whole presents an appearance which suggests that the deposit was produced by pouring fibrin-forming fluid upon the surface and causing its coagulation without producing serious injury to the epithelium. Having carefully observed the periphery of the pseudo-membrane and having changed to high power, the observer must carefully approach that portion of the membrane which is more firmly fixed to the deeper portions of the mucosa. As we proceed in this direction we find that the epithelium shows increasing alterations in structure. The superficial layers are missing, the deeper layers are separated and possess imperfect staining properties, or the nuclei are shrunken and fragmented. The layers of epithelium are thickly permeated with leucocytes. Many of the leucocytes are drawn out in a fashion which suggests that they were fixed in the act of penetrating between the flattened epithelial cells. Where the membrane dips down into the tissue, the epithelium is totally destroyed, or only an occasional, pale, isolated element may be seen. The fibrin at this point has lost its delicate net-like appearance and is massed together in hyaline clumps. At the margin the fibrin spreads out into fine fibers which penetrate between the tissue cells. The entire fibrin mass contains a large number of leucocytes, a great many of which have probably passed through the vessel walls at the time of exudation.

The boundary of the membrane in the tissue is very irregular, and extends for varying distances into the deeper layers of the tissue. (Plate XVIII., Fig. 1.) Under high power the tissue in which are the greatest deposits of fibrin is seen to be in an advanced state of necrosis. This may be detected by the poor staining qualities of the cells, the presence of hyaline masses and disintegrated nuclei. The presence of blood-containing vessels or remains of vessels within the necrotic mass shows that the destruction has included a portion of the mucous membrane. In adjacent portions of the mucous membrane the tissue is thickly infiltrated with leucocytes. An increase in the number of leucocytes is found in the blood vessels, which also contain masses of coagulated fibrin. The adjacent lymph spaces are likewise filled with fibrin. As necrotic changes in the tissue are usually present in this region it is impossible to say whether the coagulation of the fibrin in the vessels is an ante- or post-mortem change. The leucocytes are seldom found in the superficial layers of the membrane, generally disintegrating before they reach the surface. This breaking down and destruction of the leucocytes is usually attributed to the action of the soluble poisons of the organisms. When the pseudo-membrane is properly stained (Gram or Weigert preparations) more or less extensive masses of organisms are found in the superficial portions of the deposit. More rarely these appear in the deeper layers of the membrane or in the tissue. Under high power the masses are resolved into bacteria of different size and among them occasional groups of small, slightly curved bacilli with enlarged and deeply stained

PLATE XV.



ends. These are the diphtheria bacilli. (Plate IX., Fig. 8.) The other organisms are usually non-pathogenic organisms which invade the membrane and, after the exodus, proliferate to such an extent as to more or less obscure the specific bacilli. A more confusing form of bacillus which can only be recognized from the true diphtheria bacillus by cultural and staining characteristics, is the so-called pseudo-diphtheria bacillus.

From what has been said we may obtain a fairly definite idea of the diphtheritic process. This consists of destruction of the epithelial covering and underlying structures of the mucous membrane, with exudation of fibrin from the blood vessels, both due to the action of the diphtheria bacillus. The exudate permeates the injured tissue cells, which take on hyaline degenerative changes, forming thick hyaline masses. These masses, with the coagulated fibrinous exudate, form the pseudo-membrane.

In Plate XIV., Fig. 6, a portion of the exudate rests upon a distinct membrana propria. This indicates that the exudate has occurred just at the opening of the larynx, where the epithelium changes from squamous to cylindrical and is supported by a basement membrane. A similar condition is found when the deposit penetrates into the trachea and bronchi and may well be considered under a separate heading.

B. **Diphtheria of the Trachea and Bronchi.**—The pseudo-membranes of the trachea and bronchi are not as adherent as those of the pharynx and may be readily stripped from the bronchus, leaving a reddened, but smooth surface. *The fresh examination of the membrane* gives the same results as in the pharynx, except that the network shows a greater proportion of fine fibrin threads and fewer hyaline clumps. The process is best studied in hardened tissues the sections being laid transversely through an unopened bronchus.

M. F., F. M., picrocarmine, van Gieson or Weigert's fibrin stain.

The membrane at once attracts attention and consists of fine threads of fibrin and many leucocytes. As a result of the hardening process it is often retracted and does not rest directly upon the mucous membrane, except where the mucous glands open upon the surface. (Plate XV.)

By higher magnification we observe that the epithelium of the bronchus is missing, at least where the membrane is well formed, and that the membrane does not send projections into the tissue as in the pharynx, but is separated from the structures beneath by a distinct, highly refractive membrane, the basement membrane, upon which normally rests the cylindrical epithelium of the

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DESCRIPTION OF PLATE XV.

Pseudo-membranous trachitis. Tricolor photomicrograph. See page 161. Very low power. (Micro-collinear.) Hæmatoxylin alizarin. The mucous membrane is supported by a horseshoe of cartilage. Above two enlarged lymph nodes. Within the horseshoe the structure of the mucous membrane with closely packed mucous glands. The membrana propria of the mucous membrane is distinctly visible. The epithelium is missing. Resting upon the surface of the membrana propria and extending into the openings of the mucous glands a thick pseudo-membrane. The deeply-stained portions of the membrane consist of fibrin. The outer lightly stained layers which extend into the mucous glands are composed of a mixture of fibrin and mucus. At the open portion of the cartilaginous structure a band of muscle connecting the ends. Adjacent blood vessels markedly hyperæmic (*magnifier*).

bronchus. The penetration into the deeper structures of the toxines produced by the diphtheria bacillus, is largely prevented by the membrana propria, which is able to withstand the action of the organism for a greater period of time than the epithelial covering of the upper air passages. Likewise the fibrin mass may not penetrate into the tissue and the membrane is not as securely fixed as in the pharynx. In the larynx the character of the pseudo-membrane is dependent upon the structure of the mucous membrane. Where the deposit occurs upon the portions which are covered with squamous epithelium, as the vocal cords, the membrane is usually firmly fixed and possesses the appearance and character of the pseudo-membranous deposits found upon the soft palate. In the other portions of the larynx the membrane partakes more of the character of that found in the trachea and bronchi. Even the mucous membranes, which are provided with a basal membrane, are not proof against the more serious action of the bacilli. In some cases the toxic action of the organisms may extend beneath the basement membrane and when this leads to necrosis, the membrane may become as firmly fixed as in the portions of the pharynx which are covered with squamous epithelium.

C. Enteritis Pseudo-Membranacea.

Structure.—We distinguish three coats in the wall of the small intestine: The mucosa, the submucosa and the muscularis. The mucosa of the small intestine is thrown into more or less regular transverse folds, the villi. These are supported by a connective tissue structure and are covered by the typical intestinal epithelium which is of the high cylindrical type. Between the specific epithelium are a large number of mucous goblet cells. Around the base of every villus are a number of depressions lined with cylindrical epithelium. These are the crypts of Lieberkühn. In the axis of every villus is a well-developed lymph vessel system. The mucosa is separated from the submucosa by a fine layer of muscle fibers, in which may be distinguished a circular and longitudinal coat (muscularis mucosa). The muscularis mucosa follows all of the elevations of the surface. In the mucosa are found a large number of lymphoid patches which extend upward nearly to the surface and downward through the muscularis mucosa and into the submucosa. Directly over these lymphoid patches, the so-called Peyer's patches, there are no villi and the glandular structures are pushed to one side. The muscularis of the intestinal wall consists of an inner circular and an outer longitudinal coat. Between these lies the so called Plexus Mesentericus (Auerbach), which consists of a network of ganglion cells belonging to the sympathetic system. These may be detected by low power, as long, slender bodies lying between the muscle fibers. When examined under high power these bodies are found to lie in clefts in the tissue which closely resemble the lymph spaces. The nuclei are large, spherical or vesicular in form. The cell protoplasm is not uncommonly drawn out into processes (ganglion cells). The ganglion cells are sometimes surrounded by similar cells which are flattened. The typical position and number of these cells should prevent the observer from confusing them with a possible invasion of the lymph spaces by carcinomatous cells. The muscularis is covered by a connective tissue layer which forms the serosa and this contains a large amount of elastic tissue and is condensed at the surface into a membrana limitans. Upon this rests a single layer of flattened polygonal epithelium. The mucous membrane of the large intestine is free from villi but contains a much greater number of deep crypts of Lieberkühn than the smaller intestine. These are likewise richer in goblet cells. The superficial covering of the mucous membrane consists of the characteristic cylindrical epithelium of the intestine. The remaining structures of the wall of the large intestine are similar to those of the small intestine with the exception that the outermost longitudinal muscular coat is incomplete and consists of three narrow bands which are shorter than the other layers of the intestine, so that the intestinal wall is thrown into numerous folds which project into the intestinal lumen (plicæ sigmoides).

The structures about the lymphoid patches in the large intestine are differently arranged than in the small intestine. The lymph nodes lie deeper, being found at the boundary of the mucosa and submucosa. Most of them, however, lie in the latter. At these points the muscularis mucosa is interrupted. The mucous membrane covering the follicles contains the usual glandular structures and those at the center

PLATE XVI.

Fig. 1.

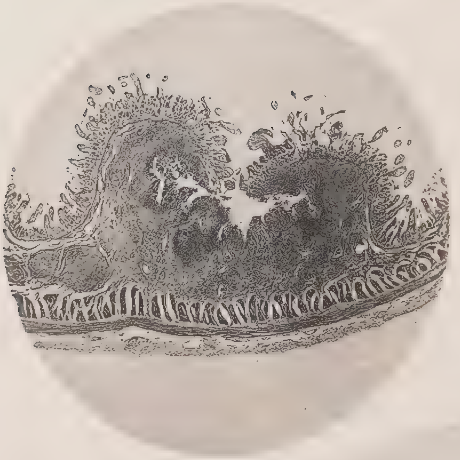


Fig. 2.

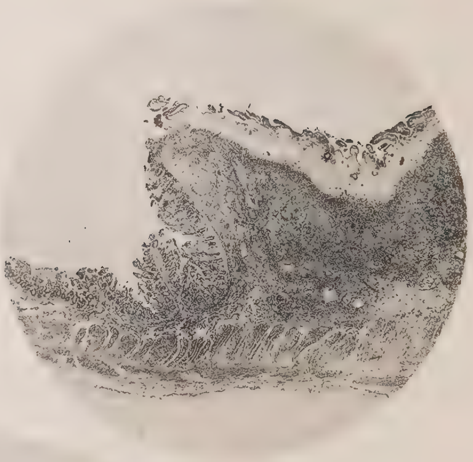


Fig. 3.

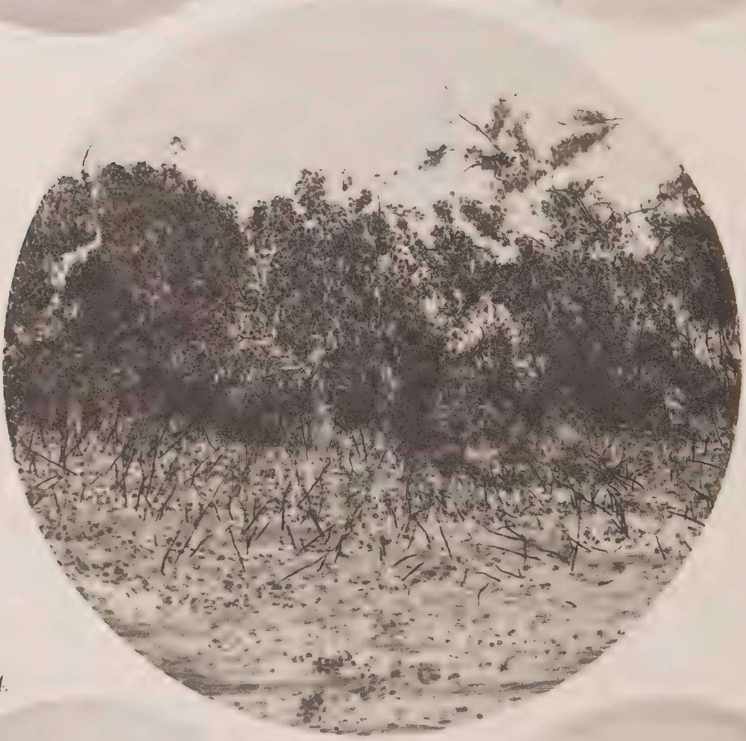


Fig. 4.

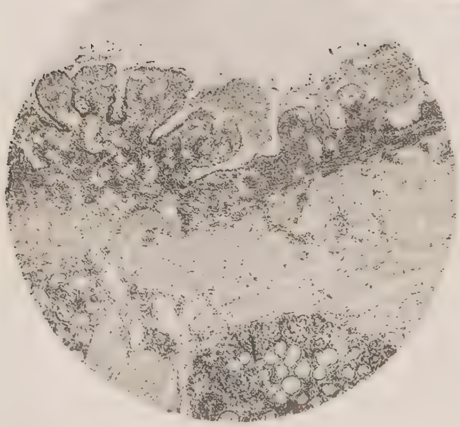


Fig. 5.

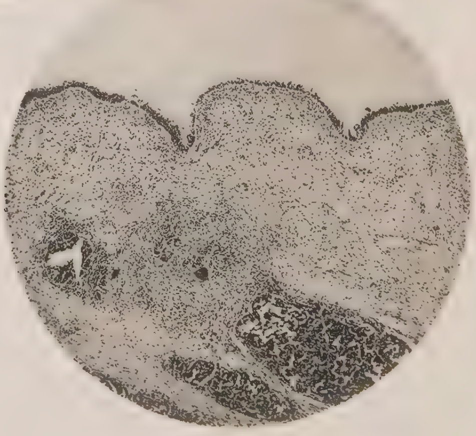


PLATE XVII.

Fig. 1.

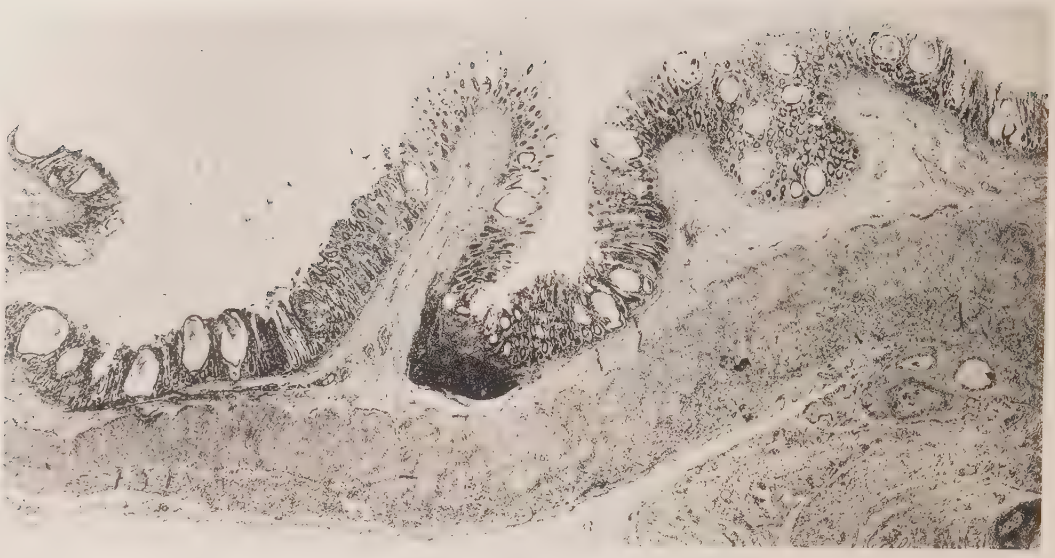


Fig. 2.

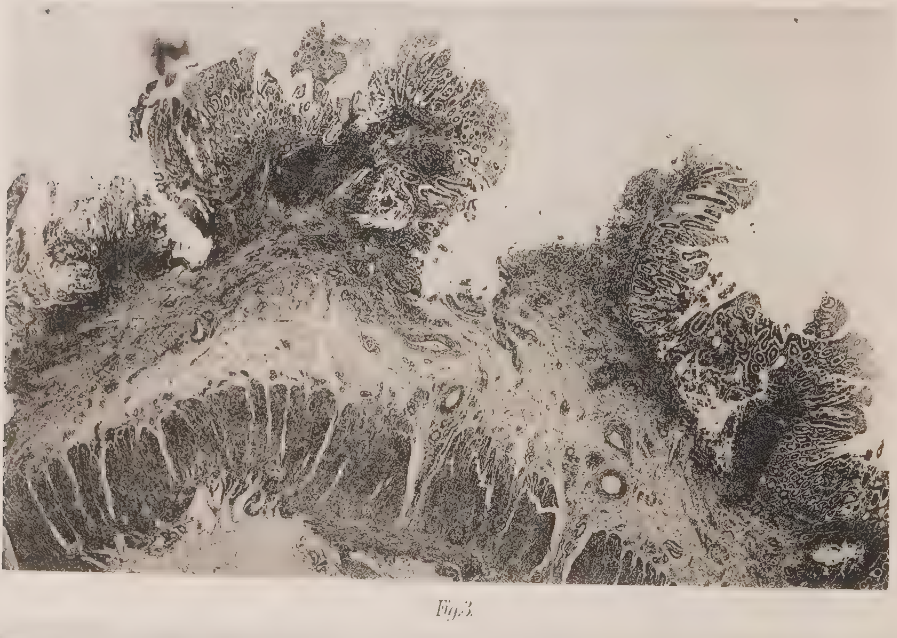
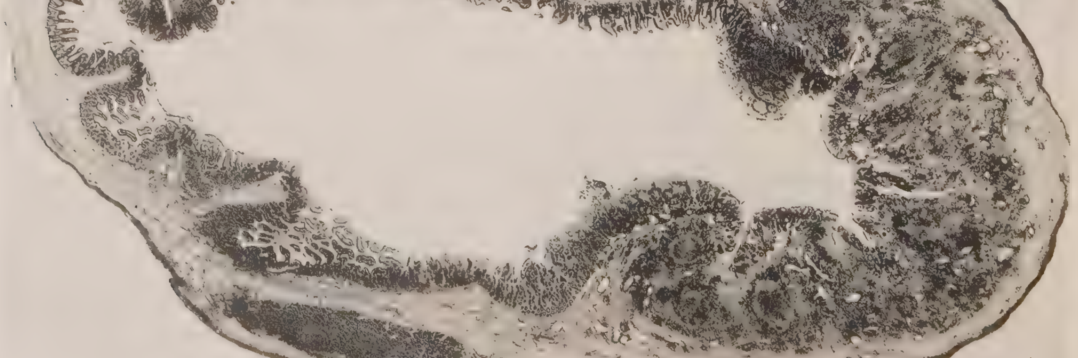


Fig. 3.



of the node dip down into the lymphoid tissue, and terminate in the region of the muscularis mucosa. This dipping down of the mucous membrane over the lymphoid patches may be detected with the naked eye.

Pseudo-membranous deposits appear on the mucous surface of the intestinal tract in severe infectious catarrh, following fæcal impaction, strangulation of portions of the intestinal wall in uræmia, as a result of poisoning with sublimate or arsenic, and as a subordinate feature of the dysenteric process. The deposits are most commonly localized upon the folds of the large intestine. In the beginning of the process these may be washed from the reddened intestine and be detected as minute flocculi swimming in the water. In some severe cases the apices of the folds are covered with dirty gray, bloody or yellowish crusts which later in the process coalesce. The remaining portion of the mucous membrane is excessively swollen and dark red or reddish black in appearance.

For microscopic examination sections should be made in the long axis of the intestinal wall and should include, if possible, some of the smaller deposits.

M. F., F. M. or embed, picrocarmine or van Gieson or hæmatoxylin eosin. F. or F. A., embed, fibrin stain. When examined under low power the apices of the folds are seen to be covered with cap-like masses, which are occasionally adherent to each other. Similar deposits are found upon the sides of the folds. All consist of fibrin, mucus and degeneration products of the epithelium. The poor staining qualities of the epithelium in the neighborhood of these masses show that necrosis has occurred. It must not, however, be forgotten that the epithelium of the intestinal tract is especially prone to post-mortem change, and that the material must be fresh. The remaining portions of the mucous membrane are hyperæmic and richly infiltrated with leucocytes. The

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DESCRIPTION OF PLATE XVI.

FIG. 1. Tuberculous ulcer in the small intestine. See p. 164. Very low power. Hæmatoxylin. Below the serosa of the intestine. Above this the longitudinal coat upon which rests the circular coat in cross section. The submucosa and mucosa are greatly thickened. At the center is an excavation about the margin of which the mucosa and submucosa produce marked elevation of the surface which is covered by the epithelium of the intestine. The whole forms a characteristic ulcer, the edges of which are undermined and overhang the excavation. The deeply-stained portions of the ulcer consist of caseous material. To the left of the ulcer three characteristic tubercles, one of which is bisected by the margin of the figure.

FIG. 2. Typhoid swelling of the intestine. (Beginning necrosis.) See p. 166. Very low power. Hæmatoxylin. To the left unaltered intestine. To the right excessive thickening of the submucosa, the surface of which is necrotic (pale). On the surface may still be recognized the structure of the villi.

FIG. 3. Aphthæ of the œsophagus. See p. 174. High middle power. Alum carmine. Gram. At the surface a thick pseudo-membrane composed of leucocytes, desquamated epithelium and the filaments of the fungus. The filaments of the organism extend well into the muscular wall of the œsophagus where they are surrounded by accumulations of leucocytes.

FIG. 4. Bronchitis (subacute). See p. 156. Low power. Hæmatoxylin. Below and to the right a group of mucous glands. Above these a thick layer of non-striated muscle fibers. Above this the submucosa thickly infiltrated with cells. The mucosa projects into the lumen of the bronchus in papillary-like projections. Upon the surface of the mucosa a mixture of cells and mucus. The papillary projections contain engorged capillaries within which may be seen a large number of leucocytes.

FIG. 5. Tuberculosis of the larynx. See p. 158. Low power. Hæmatoxylin. Above the surface of the larynx from which the epithelium is desquamated. To the right and below groups of mucous glands deeply stained. To the left the excretory duct of the mucous gland, cross section. Between this and the mass of glands to the right, a tubercle with three giant cells.

dilated lymph spaces contain more or less extensive deposits of fibrin. In severer cases of intestinal pseudo-membranous inflammation, such as result from sublimate poisoning, the entire mucosa becomes necrotic. (M. F., F. M., N. S., methylene-blue, F. or F. A., F. M., fibrin stain.) On examination under low power the superficial layers of the membrane are found to be in a state of complete necrosis. In the deeper layers occasional remains of the glands may be detected. The vessels of the submucosa are markedly injected and minute hemorrhages into the tissue are common. At the periphery of the necrotic area is seen an extensive accumulation of cells. Under high power these are found to be polymorphonuclear leucocytes. Those directly adjacent to the necrotic area show the already described disintegration of nuclei and karyorhexis. Sections stained with Weigert's fibrin method disclose in the necrotic areas an unexpected amount of fibrin in the lymph channels of the submucosa. The necrotic portions of the tissue contain enormous numbers of bacteria of all forms. As all of the intestinal bacteria do not stain with Gram's method (Weigert) a control must be made by staining other sections with methylene-blue. In many cases of pseudo-membranous inflammation (dysentery) a long, fine bacillus is found, which penetrates into the portions of the tissue which are not necrotic. As this organism is not accompanied by the saprophytic organisms which invade the necrotic mass, it is very probable that it is the exciting cause of the forms of dysentery in which it is found. (See Dysentery.)

4. Tuberculosis of the Intestine.

The ingestion of milk containing tubercle bacilli, or the swallowing of sputum containing the same organism by patients suffering from phthisis pulmonum may lead to a tuberculous infection of the intestine. As fæces are most likely to accumulate just above the ilio-cæcal valve, the lower end of the ilium is the locality most often infected. The infection takes the form of ulcerated patches having their greatest diameter transverse to the long axis of the intestine. These ulcers present a characteristic appearance, having rough, irregular, protruding edges, often undermined, and a chopped out and roughened floor in which minute, tubercle-like points of caseation may be seen. The investigation of the serous surface directly beneath the ulcer offers an important aid in the determination of the character of the process. In tuberculous ulceration rows of minute tubercles may often be observed beneath the serous covering of the intestine (tuberculosis of the lymph vessels).

Beside the patches of ulceration in the intestine, isolated tubercles may be found in the mucous membrane, which may only be differentiated from the swollen solitary lymph follicles by their size and the presence of caseation. These solitary tubercles are found in great numbers in Peyer's patches, where they form a stage in the formation of the ulcers.

A small ulcer should be hardened *in toto* and sectioned. M. F., F. M., N. S. The microscopic examination of the isolated tubercles shows that they are located in altered lymph nodes. These appear enlarged and are pale at the center. Under high power the enlargement of the lymph node is found to be due to an increase in lymphocytes. In the center of the node are found the characteristic arrangement of epitheloidal cells, giant cells and caseation, which mark the formation of tubercles. When the caseation extends to the surface we have the beginning of an ulcer. The characteristic appearance of the ulcer may be best observed with low magnification. The overhanging edges are covered by the epithelium of the mucous membrane, and surround the excavation which extends through the submucosa into the muscularis. (Plate XVI., Fig. 1.)

Areas of caseation and disseminated miliary tubercles with giant cells are to be seen at the periphery and in the floor of the ulcer. The coalescence of these tubercles and the gradual loosening of the caseous material causes constant enlargement. Breaking down of the miliary tubercles gives the roughened aspect to the floor and sides of the ulcer. In favorable sections the rows of miliary tubercles lying directly under the serosa may be observed. In rare cases the surface of the ulcer is covered with granulation tissue which may or may not contain giant cells, or areas of caseation. Properly stained sections usually show large numbers of tubercle bacilli in the caseated material or the more rare granulation tissue.

5. Typhoid Infection of the Intestine.

(Typhus abdominalis.)

The immediate result of infection of the wall of the small intestine by the typhoid bacillus is swelling of the lymphoid tissue, especially the patches of Peyer (first stage).

Necrosis beginning at the intestinal surface and extending to the deeper structures of the lymph nodes, marks the so-called second stage of the process. The disintegration and removal of the necrosed material produces areas of ulceration which, in contradistinction to tuberculous ulcers of the intestine, are placed with their greatest diameter in the long axis of the intestine, coinciding in form and distribution with the patches of Peyer (third stage).

These ulcers have smooth perpendicular edges, and, when the necrotic material is removed, relatively smooth bases.

A fourth stage follows upon the three just given, and consists of the establishment and completion of repair by the formation of scar tissue.

The first and second stages of the process are most characteristic and specimens from each of these periods of the disease should be chosen for study.

M. F., F. M., N. S. or Löffler's methylene-blue. F. A., F. M. or embed, fibrin stain.

(*a*) **Lymphoid Swelling.**—In sections taken from the normal intestine the patches of Peyer consist of accumulations of lymphocytes in the mucosa.

At the points where these accumulations of cells occur the mucosa is markedly thickened, for the most part at the expense of the submucosa, the muscularis mucosa being displaced toward the submucosa and except in the larger patches, sharply delimiting the lymph node at its base.

Some of the larger patches penetrate through the muscularis mucosa into the submucosa.

All of these lymphoid accumulations in the normal intestine have sharply defined boundaries, so that even by low power they may be easily distinguished as definite structures.

Turning to the inspection of a section through a swollen Peyer's patch from an intestine infected with the typhoid bacillus, we find that the sharp delimitation of the node is obliterated.

The patch is no longer bounded by the muscularis mucosa, but penetrates into and through the submucosa, often invading the outer muscular coats. (Plate XVII., Fig. 3.)

The marked increase in the lymphoid tissue elevates the mucosa, which in cross section appears stretched over the surface of the swelling.

By higher magnification the swelling of the patch can be traced to an increase of cellular elements, a large proportion of which are the lymphocytes which formed the original lymphatic structure.

Beside these are a large number of spindle-shaped cells with spherical nuclei especially about the capillaries and smaller vessels. These cells are derived in part from the connective tissue structure of the node, and the endothelium lining the lymph spaces, with possibly a few migrating cells of special type. A large proportion, increasing as the process advances, of the newly-formed cells are polynuclear leucocytes. We thus see that under the irritative action of the typhoid bacillus, a type of granulation tissue has been formed.

(*b*) **Necrosis.**—The necrotic portions of the patches of Peyer may be readily recognized by the absence of stained nuclear structures. (Plate XVI., Fig. 2.)

Sections made perpendicular to the axis of the intestinal canal, including the infected patch, enable us to observe the form and extent of the necrosed area.

The surface of the patch shows greater involvement of the tissue by the necrotic process than do the deeper portions, the extent of the necrosed area gradually decreasing as we proceed downward, giving the necrosed tissue the form of a blunted cone with the base at the surface of the patch. At the periphery of the necrotic area, even when of great extent, a zone of cell infiltration and proliferation mark the boundary between dead and living tissue.

The necrotic process often extends to the muscularis, occasionally into it, and in very rare cases through the muscular coats, causing perforation of the intestinal wall. Frequent spots of hemorrhage mark the boundary of the necrosed tissues. The blood vessels about and extending into the necrosed mass, appear dilated and engorged, and contain well-conserved red blood globules and leucocytes, with no evidence of coagulation.

The frequent hemorrhages which follow separation of the sloughing patches are due to the condition of the blood vessels just noted. By staining sections with methylene-blue, we may determine the presence of deep blue masses near the surface of the patch, which under high power are resolved into groups of cocci and larger and smaller bacilli, organisms which have merely invaded the necrosed tissue. In the deeper parts groups of a fine slender bacillus will be found which is usually the specific organism, the typhoid bacillus. The absolute proof of the identity of the organism may be obtained only after cultivation, because of the similarity in appearance of the numerous bacilli of the colon group.

(*c*) **Ulceration and Repair.**—M. F., F. M., N. S., or elastic fiber stain. If the floor and edges of a clean ulcer be examined under the microscope the tissue at the periphery of the ulcer will be found richly infiltrated with cells. At a later period a vascular granulation tissue develops from these cells and covers the denuded surface. When this granulation tissue becomes transformed into fibrous connective tissue the epithelium from the edges usually proliferates and closes in the defect. A regeneration of the characteristic structure of the epithelium covering of the intestinal wall does not occur, although here and there villus-like projections or glandular depressions may be detected. The destroyed lymphatic structures are not restored.

6. Dysentery.

Dysentery, a process affecting primarily the large intestine, is probably not a disease with a constant etiology, but a more or less characteristic pathological process induced by different agents. The affected intestine shows areas of ulceration and irregular patches of diphtheritic deposit. The curious and irregular distribution of the ulcers gives to the intestinal surface an appearance not unlike that of a chart or map. These often undermine the mucosa and lead to the separation of large pieces of membrane. Partially detached portions of the membrane may be encountered in sections where the mucous membrane is stripped from the muscular coats. At the termination of the process the repair of the injured and denuded surfaces leads to the formation of extensive patches of scar tissue.

Formaline, F. M., N. S. or van Gieson or methylene-blue. F. or F. A., F. M. or embed. Fibrin stain.

Many cases of the affection present alterations of the mucous membrane identical with those described under pseudo-membranous inflammation of the intestine. The ulcerative portion of the process presents features of interest. On examining cross sections of the affected intestine with low power, the undermining and erosion of the mucosa is at once apparent. The greatest amount of erosion is found in the deeper layers of the mucosa and extends into the submucosa causing the superficial covering of the membrane to overhang the edges of the excavations. Where the denudation is extensive, isolated mushroom-like projections of the mucosa, supported upon stems dot the eroded surface. (Plate XVII., Fig. 2.) The entire mucosa and submucosa in the regions adjacent to the ulcers is infiltrated with cells, for the most part, polymorphonuclear leucocytes. The purulent character of the process is likewise shown by the large number of pus cells and mucus found in the evacuations of patients.

In the bases of the ulcerated areas an interesting phenomenon is occasionally observed, which consists of a more or less complete covering in of the floor of the ulcer with epithelium, even when the ulceration has extended into the deeper layers of the submucosa where epithelial structures or glands do not exist. By tracing the newly-formed epithelial covering to the edge of the excavation it is found to be continuous with the intestinal epithelium. The occurrence of proliferation in these cases should not be accepted as an attempt at regeneration, but is more probably the result of direct stimulation of the epithelium. Proliferative changes are found in the epithelium covering the follicles, where the muscularis mucosa is interrupted and the space is filled in with lymphoid tissue. Here the glands of the mucous membrane extend deeper into the submucosa than normal and are not uncommonly branched or show cystic dilatation. When the process subsides the defects in the mucosa are repaired by granulation tissue which is later transformed into fibrous connective tissue.

As stated at the beginning of this paragraph, the process is not always occasioned by the same agent and does not possess a fixed etiology. In many cases an animal parasite, the amœba coli, has been deemed the specific cause of the disease.

REFERENCE:

Councilman and Lafleur. Amœbic Dysentery. Johns Hopkins Hospital Reports, Vol. II., 1891.

DESCRIPTION OF PLATE XVII.

FIG. 1. Colitis cystica. See page 168. Very low power (micro-collinear). Hæmatoxylin. Cystic dilatation of numerous glands. Beneath the epithelium near the center of the preparation is an enlarged lymph node, which extends into the submucosa.

FIG. 2. Dysentery. Section of colon. See page 167. Very low power (micro-collinear). Hæmatoxylin. Below the circular muscular coat of the colon in cross section. Above this the pale submucosa. At the center a deep ulcer. To the left and right smaller ulcerous excavations, leaving in the center a mushroom-like projection of the mucosa. The ulcers extend well into the submucosa.

FIG. 3. Typhoid swelling in the intestine of a child. (Tenth day of disease.) See page 165. Very low power (micro-collinear). Hæmatoxylin. (Intestine is cut somewhat obliquely and is unopened.) To the right marked swelling of the mucosa and submucosa. Included within the thickened portion below, the structure of the lymph nodes may be plainly seen. To the left of the figure the mesenteric attachment of the intestine.

This organism has much the appearance of an isolated cell, possessing a nucleus. In fresh preparations on a warm stage it shows marked amœboid movement. It is from 15–25 μ in diameter and is found in the tissue surrounded by pus corpuscles, forming minute or larger abscesses. It is likewise found in the widely dilated lymph spaces of the submucosa. By special methods (Mallory) the nucleus of the organism may be stained red, its protoplasm blue. The form of dysentery encountered in the tropics appears to be produced by a bacillus described by Shiga. This organism is about the thickness of the colon bacillus, of variable length, with slightly rounded ends and does not stain with Gram's method. It is sluggishly motile and gives a Widal reaction with the blood of the patient from which it is obtained; that is, the organism, when subjected to the action of the blood serum of the patient, ceases motion and by a process of agglutination forms in clumps.

7. Colitis Cystica.

In chronic inflammatory conditions of the large intestine the surface of the colon may be studded with minute, barely visible, clear vesicles. (*F.*, *F. M.*, *N. S.*) Sections of the tissue examined under low power show the character of the vesicles, which are produced by dilatation of the gland lumenæ, the openings of which have become occluded. Chronic irritation of the surface of the mucous membrane in the large intestine commonly causes agglutination of the openings of the glands, followed by secretion into the closed lumen, which results in the formation of small oval or spherical cysts. These project above the surface producing the vesicular appearance noted in the macroscopic specimen. (Plate XVII., Fig. 1.)

8. Amyloid of the Intestine.

In widespread amyloid change the intestine is not uncommonly involved. The presence of amyloid is detected by treating the mucous surface of the intestine with Lugol's solution when the intestinal villi take the usual brown coloration which gives the surface a dotted appearance. A portion of the intestinal wall should be removed with a curved shear or sections should be made with the double knife and the material thus obtained stained with Lugol's solution (wash in water, embed in glycerine). Under the microscope the villi show a marked brown coloration without disclosing in which structures the amyloid is deposited. In the submucosa, however, the amyloid material is found to be deposited in the walls of the blood vessels.

9. Melanosis of the Intestine.

In cases of chronic intestinal catarrh a black discoloration about the periphery of the lymph nodes or more rarely involving the entire villi may be encountered (Zottenmelanose).

The material for *fresh examination* may be obtained with a curved shear. Under the microscope the tissue is found to contain a more or less uniform and extensive deposit of irregular, dark granules, which, with the exception of their color, present the characteristic appearance of blood pigment. The dark coloration of the pigment in these cases is probably due to a chemical combination between the iron of the pigment and the sulphuretted hydrogen in the intestinal tract (H_2S). On the addition of hydrochloric acid the characteristic brown and yellow color of the pigment is restored.

REFERENCE:

Flexner. The Etiology of Tropical Dysentery. Johns Hopkins Hospital Bulletin, Vol. XI., No. 115.

10. Hæmochromatosis of the Intestine.

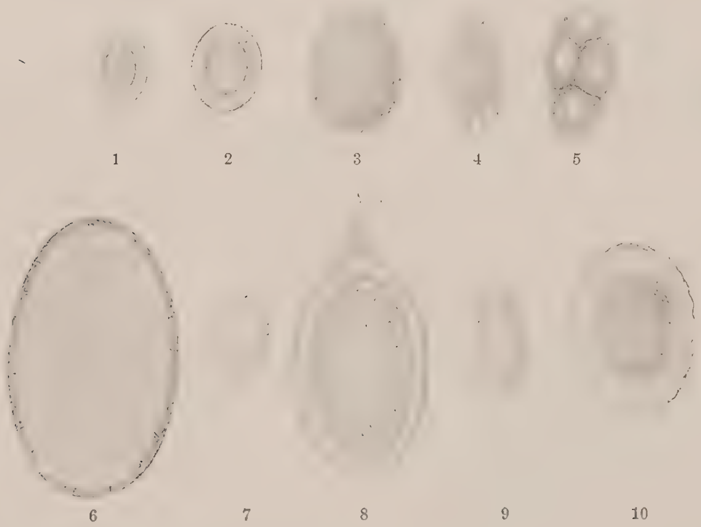
In the condition known as hæmochromatosis, the walls of the intestines share in the general pigmentation. A similar brown coloration of the intestinal wall is found in advanced age, in drunkards and in the cachectic. The examination of *fresh material* discloses extensive deposition of yellow pigment in the muscle fibers, usually in groups at the poles of the nuclei. The greater portion of this pigment gives a negative reaction for iron and is similar to the autochthonous pigment of the heart muscle and liver cells.

11. Intestinal Parasites.

Excreta from the infected intestinal tract naturally contain the organisms which produce the disease. For instance, typhoid bacilli, tubercle bacilli, amœbæ coli, bacilli of tropical dysentery, cholera bacilli. In many cases these organisms may be easily detected in fresh (amœbæ) or stained cover-glass preparations (cholera bacilli, tubercle bacilli). The amœbæ when not showing amœboid movement are usually round, nucleated bodies (24 μ).

The protoplasm of the amœbæ has a high refractive index and the whole presents a curious glistening appearance. When observed in the living state, especially upon a warm stage, they show more or less active amœboid motion, running out short, rounded pseudopodial projections. The more or less spherical nucleus is very readily detected when acetic acid is added to the preparation. The protoplasm occasionally contains fragments of red and white blood corpuscles. Fat droplets are usually absent. The nuclei of the amœbæ are not stained with the ordinary nuclear stains.

Fig. 21.



Ova of intestinal parasites (uniform magnification). 1, Tænia solium (32–35 μ long); 2, T. saginata (40–45 μ long); 3, Bothriocephalus latus (70–80 μ long); 4, Trichocephalus dispar (52–60 μ long); 5, Anchylostoma duodenale (58–62 μ long); 6, Distomum hepaticum (130–145 μ long); 7, Distomum lanceolatum (40 μ long); 8, Distomum hæmatobium (120 μ long); 9, Oxyuris vermicularis (52–55 μ long); 10, Ascaris lumbricoides (60–70 μ long). (Collected from Orth's Pathology, Heller in Ziemssen's Handbook, and Bizzozero, Clin. Microscopy.)

Reference:

Goebel. Virchow's Archiv, Bd. 136, 1894.

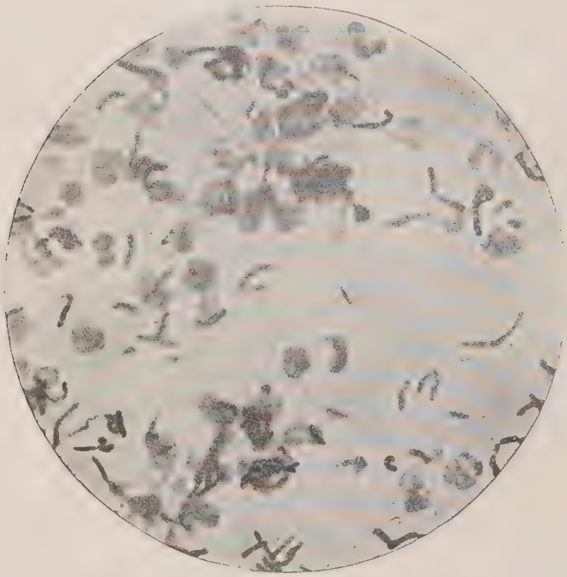
The similarity between the typhoid bacillus and those of the colon group which normally inhabit the intestinal tract, prevents a differentiation between these organisms without the aid of elaborate culture methods.

Beside these minute organisms the intestine is not uncommonly infected by larger parasites, usually worms. The presence of these parasites usually leads to more or less indefinite symptoms, the principal of which are anæmia and disturbance of the intestinal function. Their presence may be occasionally detected by the passage of segments or complete worms with the fæces. A further method by which the presence of worms in the intestinal tract may be determined is the investigation of the excreta for the embryos of these parasites. These are frequently present in great numbers and may be differentiated by their characteristic form and difference in size. (Fig. 21.)

12. Anthrax of the Intestine.

In rare cases the intestinal tract is the site of infection for the bacillus ot anthrax. This organism produces areas of hemorrhagic infiltration, pseudomembranous deposits and purulent ulcerating excavations, the so-called mucous carbuncle. The site of its action is usually the small intestine. The microscopic appearances are especially interesting, because of the large number of organisms and the facility with which they may be demonstrated.

FIG. 22.



Phagocytosis in anthrax of the intestine. Fragments of anthrax bacilli are seen within the bodies of leucocytes. (Gram, oil immersion.)

M. F., F. M., or embed. Stain after Gram.

The connective tissue beneath the epithelium is markedly swollen and œdematous. The lymph spaces are greatly widened in the mucosa and submucosa and filled with masses of large, deeply stained bacilli. (Plate XIX., Figs. 1 and 2.) Masses of bacilli may likewise be found in the lumenæ of the intestinal glands and between the epithelium, thus demonstrating the route which they have taken in the invasion of the tissue. Beside œdematous infiltration the earlier foci are marked by an excessive accumulation of leucocytes (Plate XIX., Fig. 2), especially in the submucosa where they infiltrate the masses of bacilli. The anthrax bacillus is a large and thick rod which grows into long filaments or may appear in the form of short fragments. Careful examination of the leucocytes which surround and infiltrate the masses of bacilli, shows that a certain number of them contain fragments of the organisms. The protoplasm of some of these leucocytes is thickly packed with the broken-down organisms and as the nuclei of the cells show no change, it is fair to assume that the leucocytes have destroyed both the virulence and the vitality of the bacteria (phagocytosis). (Fig. 22.) Whether or not the leucocytes are the active agent in the destruction of the organisms, the fact remains that they take up in their protoplasm the remains of the organisms which they transport to distant parts and possibly digest. The subsequent processes of necrosis, purulent demarkation and the formation of ulcers in anthrax of the intestine, are not sufficiently characteristic to require special description.

13. The Vermiform Appendix.

Structure.—The mucous membrane of the vermiform appendix differs from that of the other portions of the intestinal tract in the amount of lymphoid tissue which it includes. This may be in the form of lymph nodes or patches, but very commonly the nodes coalesce forming a complete circle of lymphoid tissue about the lumen of the appendix.

A portion of the glandular tissue which is found during fœtal life in the vermiform appendix disappears before birth after undergoing a transition into cysts (Stohr). In adults the glandular tissue has totally disappeared and in advanced age a senile atrophy of the mucous membrane, and diminution in the size of the lymph nodes occurs.

F., F. M., N. S.

The vermiform appendix is commonly affected by stricture or alteration of the lumen.

According to Ribbert a careful distinction must be observed between a physiological obliterating process beginning in the distal end of the organ, and atresia of the lumen following inflammation at or near the junction of the appendix and cæcum.

In the first named process the obliterated portion of the appendix consists of a center of connective tissue containing a few vessels radiating toward the center and surrounded by the muscular coat, the muscularis mucosa having disappeared. This central mass of connective tissue represents the remains of the submucosa, a fine white dot or line of thin connective tissue fibrils marking the location of the original mucosa.

The adjacent mucous membrane is atrophic and thin, the glands are obliterated and the surface is covered by a single layer of cylindrical epithelium. In inflammatory stenosis of the appendiceal lumen the deposits of connective tissue within the muscular coat are more irregular and more vascular than in the physiological obliterative process above described.

The muscularis and serosa are permeated with round cells which lie in the lymph spaces between the muscle fibers and beneath the peritoneal covering.

The muscular ring is occasionally broken in its continuity and the space filled by a collection of round cells, at the same time the adjacent serous covering is found in a state of active inflammation, even when perforation of the appendix has not occurred. Inflammation of the appendix is commonly produced by stasis of fæces and the formation of fæcal concretions in the appendix, the invasion of the appendix by organisms and by the action of other agents, the exact nature of which is not known. In extensive tuberculosis of the intestines the appendix is occasionally involved. A few cases of actinomycosis of the appendix have been described. The wall of the appendix in these cases shows simple inflammatory changes, the organism being first detected in the perityphlitic accumulation of pus.

References :

Ribbert. Virchow's Archiv, Bd. 132, 1893.
Letulle und Weinberg. Archives des sciences médicales, Bd. II., 1897.

14. Erosion of the Mucous Membrane of the Stomach.

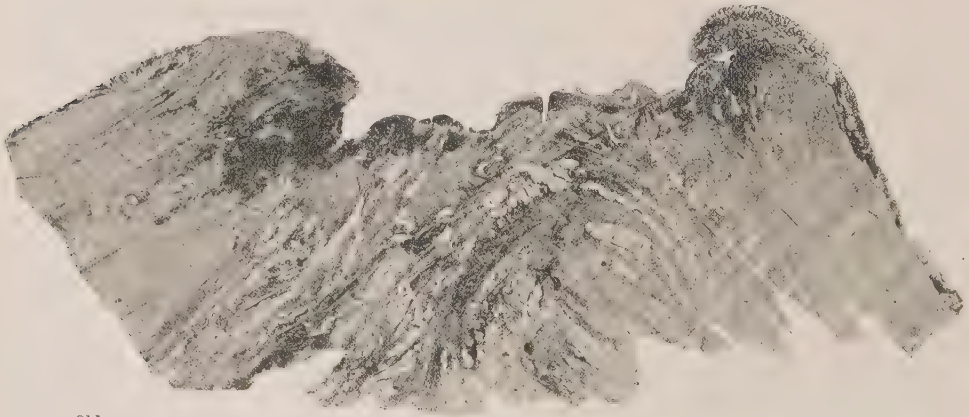
The histological structure of the gastric wall is in many respects similar to that of the other portions of the intestinal tract. Its outer aspect is covered with a serous membrane. It possesses a thick layer of irregularly arranged bands of muscle, a submucosa which is poor in cells and a more or less well-defined muscularis mucosa which separates the submucosa from the mucosa. This last is made up of the specific gastric glands and contains a variable number of lymph nodes which are placed directly above the muscularis mucosa. The surface of the membrane, beside being thrown into folds, is covered with grooves or depressions into which empty the gastric glands. These and the lumenæ of the glands are lined with the specific columnar epithelium which covers the mucous surface. The columnar epithelial cells of the stomach differ from those of the intestine by not possessing so wide a cuticular layer and not changing into goblet cells. Within the protoplasm of the gastric cells a mucoid substance which does not stain with the ordinary reagents, may be detected. In the greater number of the peptic glands two forms of epithelial cells may be demonstrated. Those which lie next to the lumen are cylindrical in form and are the chief or central cells. These are the greatest in number and are identical with the columnar epithelium covering the other portions of the membrane. Pressed outward from these cells and lying directly against the basement membrane are a greater or smaller number of deeply stained elements, the so-called parietal or acid cells. The glands in the neighborhood of the pylorus contain only chief or central cells. For the demonstration of the parietal cells, sections must be stained with hæmatoxylin, followed by eosin, then with a very dilute acid fuchsin solution, then with a weak aqueous solution of Congo red. The parietal cells are stained red.

In cases of acute gastric catarrh or after long periods of vomiting, rows of minute superficial ulcers possessing a dark red ground may be found in the neighborhood of the longitudinal folds. These superficial ulcers are usually accompanied by irregularly distributed dark red spots in the adjacent mucous membrane.

M. F., F. M. Carmine or hæmatoxylin eosin or van Gieson.

The dark spots are found in sections which include them, to be due to a dense infiltration of the mucous membrane with blood. Under high power the epithelium is found to have been detached from its base, as a result of the hemorrhage which has likewise penetrated through the epithelium into the lumenæ of the glands and into the crevices between the connective tissue cells. The nuclear staining of the elevated epithelium is poorer than the adjacent cells. This loss of vitality in the cells is unquestionably the result of the hemorrhage which, by

Fig. 23.



Old gastric ulcer. Repair affected by fold of omentum. On the right overhanging gastric mucosa. (Very low power.)

separating the epithelium from its base, destroys the nutrition of the cells. The remains of these hemorrhages may likewise be found in the bases of the smaller ulcers. The excavations (so-called erosions, as they only involve the mucosa), are due to the action of the gastric juice which digests away the necrotic epithelium. The vessels of the submucosa show general engorgement, indicating that the hemorrhages are most probably the result of excessive intervenous pressure. The sides and floor of the larger ulcers have a characteristic terrace-like appearance due to the varying resistance of the different layers of the stomach wall, the destruction being greatest at the surface and smallest at the bottom. Fresh inflammatory changes are usually absent from the adjoining tissue. The tissue at the edge of the ulcer is usually badly stained, but this in most cases is due to post-mortem digestion. The epithelium commonly overhangs the edge of the ulcer and may show evidences of proliferation in some cases sufficiently extensive to cover in a portion of the excavation. (Fig. 23.) The newly formed epithelium usually shows a typical glandular arrangement. The base or sides of the ulcer occasionally show minute elevations or ridges. Under the microscope these are found to contain blood vessels, which, because of their greater resistance, protrude into the excavation. The contents of these vessels are usually in a state of partial or complete thrombosis, or a more or less extensive proliferation of the intima may be present.

15. Chronic Gastritis.

Chronic gastric catarrh may lead to marked thickening of the mucous membrane of the stomach, or to general atrophy of the same. As the gastric juice begins to digest the superficial structures immediately after the exodus, the finer changes in the epithelium can only be studied in material which has been hardened directly after death. This result may be partially obtained by filling the stomach of the cadaver through the œsophagus with sublimate, alcohol or formaline, as soon after the exodus as possible. For the investigation of less subtle changes especially those affecting the connective tissue, the ordinary hardening methods are sufficient.

M. F., F. M. or embed. N. S. or hæmatoxylin eosin.

The principal factor in the thickening of the gastric wall is seen, under the microscope, to be an increase in the connective tissue structure, the result of proliferation and leucocytic (mononuclear) infiltration, which leads to more or less extensive separation of the gastric glands. The infiltration of the connective tissue between the glands may be well demonstrated in sections made parallel to the surface of the membrane. The diffuse form of proliferation must be distinguished from a nodular form in which the lymph nodes of the mucosa enlarge and may be detected with the naked eye as minute, white dots embedded in the surface. Karyokinetic changes are sometimes present in the epithelium of the glands. In some cases the epithelium near the fundus of the gland is unusually high. The parietal cells are few or absent and goblet cells may here and there make their appearance. Some cases of chronic gastric catarrh are associated with thinning of the stomach wall which is due to atrophy and destruction of the specific epithelium of the mucosa. In place of the characteristic glands the surface of the membrane is covered with short and irregular gland-like structures lined with columnar epithelium and goblet cells, as in the crypts of the

References:

Hauser. Das chron. Magengeschwür, Leipzig, 1883.
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Gerhardt. Virchow's Archiv, Bd. 127, 1892.

intestine. In rare cases the surface of the membrane is covered with small cysts.

The acute inflammatory changes of the gastric mucosa, especially when of a purulent nature, present no especial characteristics. *Fresh double knife sections* of the gastric wall in acute gastritis and in phosphorus poisoning show an extensive fatty degeneration of the epithelium and connective tissue cells (*add potassium hydrate*).

16. Aphthæ of the Œsophagus.

The œsophagus is composed of three principal layers. The upper portion of the outer layer is made up of striated, and the greater part of the lower portion of smooth muscle fibers. These are arranged in circular and longitudinal coats. Within this is a submucosa divided by a muscularis mucosa from the papillated mucosa. The surface of the mucosa is covered with multiple layers of squamous epithelium. The wall of the œsophagus contains a goodly number of mucous glands which lie in the mucosa and submucosa. About the outlets of these glands is found a certain amount of lymphoid tissue.

Infection of the pharynx, trachea, œsophagus or stomach by fungi occurs only in patients with greatly reduced vitality. Favorable conditions are sometimes found in very reduced children or in advanced cases of diabetes. The aphthous deposit makes its appearance as a white membrane, which has, however, nothing in common with pseudo-membrane in diphtheria. The aphthous deposit is more or less intimately attached to the mucous membrane and is more or less dense or of a soft or smeary consistence. If a portion of the membrane be stripped up and *examined in salt solution*, the thinner portions when viewed under low power, show a definite coarse network of threads. Under high power, these are seen to possess a definite contour of uniform intensity, are commonly divided into segments and in many places are branched. Within the interstices between the coarse threads or mycelia, are numerous highly refractive oval and round bodies (conidia). Neither threads or conidia are affected by potassium hydrate. A certain amount of more or less degenerate desquamated epithelium likewise forms a constituent of the mass. The method by which the fungus penetrates the tissue can only be observed in sections which include the underlying structures.

M. F., F. M., embed, carmine, Gram or Weigert's fibrin stain.

Under the microscope the threads and conidia of the organism are stained deep blue. Upon the surface they form a conglomerate mass from which filaments of mycelium penetrate between the layers of the flattened epithelium of the mucosa. In advanced cases the threads may extend through the muscularis mucosa and into the submucosa. (Plate XVI., Fig. 3.) The unaffected tissue is thickly infiltrated with pus cells which, when present in sufficient number, lead to purulent softening of the tissue with the formation of extensive and deep abscesses.

VIII. THE SEROUS MEMBRANES.

The term "serous membrane" includes the lining membranes of all the larger serous cavities, *i. e.*, peritoneal, pericardial and pleural.

A serous membrane consists of a supporting ground work of connective tissue upon which is a single layer of large polyhedral cells with round nuclei. (Epithelial or endothelial cells according to different authors.)

Sections of serous membranes stained with Orcein or Weigert's elastica stain show the presence in the connective tissue ground work of a layer of elastic tissue placed

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either directly beneath the epithelium, or separated from it by a narrow margin of connective tissue fibers.

As the various pathological processes which affect the serous membranes are alike, no matter what the location, whether peritoneum, pleura or pericardium, a consideration of the changes affecting any one membrane will in general suffice for all.

1. Acute Peritonitis.

The first changes in the peritoneum in acute peritonitis are a marked injection of the vessels, and a slight stickiness without appreciable loss of lustre of the surface. These early changes are best observed in cases of abdominal operation which have proved fatal after a few hours, and upon the peritoneal surface of freshly invaginated intestine.

The fluid for the fresh examination is collected by gently passing a knife over the surface of the membrane.

This presents a cloudy appearance and under high power is found to contain large polygonal cells often in groups with small round nuclei, and a greater or smaller number of polymorphonuclear leucocytes. The last are round and somewhat smaller than the desquamated epithelial elements.

A stained slide or cover-slip will determine the presence of micro-organisms, which if in great number indicate the beginning of a purulent peritonitis, if the autopsy has been made directly after death, and the organisms cannot be traced to post-mortem contamination.

Small pieces of omentum hardened in formaline or 60-per-cent. alcohol are best suited for examination. Fragments may be cut from the hardened membrane with a shear, stained with alum carmine and examined in glycerine.

The omentum consists of an open network of connective tissue covered in the normal state by a scarcely perceptible layer of flat epithelium.

The examination of the above-mentioned fragment under high power shows the epithelium markedly swollen and prominent, the nuclei enlarged and deeply stained. Many of the cells contain multiple nuclei, often four or five and the entire cell is so enlarged as to form a kind of giant cell, which differs in appearance from the typical giant cell of tuberculosis by the nuclei being grouped together in the center of the cell, whereas in the tuberculous giant cell they are most commonly arranged in a crescent near the margin of the cell protoplasm.

2. Acute Fibrinous Pleuritis.

In the majority of acute inflammations of the serous membranes the earliest alterations of the epithelium are accompanied by deposits of fibrin. These may be so slight as to be scarcely perceptible to the naked eye. In such cases the surface of the membrane is less shiny than normal, and when stroked with the flat knife blade remains dry, and loses its brilliance. Where the deposit is greater the fibrin forms a yellowish white membrane which may be readily stripped from the surface. The fresh examination of the stripped membrane proves it to consist of fibrin, including plentiful cellular elements.

N. S. or elastica stain. F., F. A. or M. F., F. M., celloidin or paraffine fibrin stain combined with elastica stain.

For the finer examination, especially of the beginning process, sections of embedded material made perpendicular to the surface are essential.

The examination, high power, of a section from a beginning fibrinous pleuritis (or pericarditis) shows the fibrin covering the surface in a thin layer, and directly beneath it marking a distinct boundary between it and the connective tissue stroma a more or less continuous row of large cubical cells with characteristic spherical nuclei.

Plate XIX., Fig. 3. This row of cells is the original cell covering of the membrane in its original position as may be seen on the examination of a section stained with elastica stain when the layer of elastic fibers will be found directly beneath the rows of cells. The fibrin is deposited upon the surface from fibrin-forming fluid which exudes from the superficial capillaries. As a result the epithelial layer is frequently raised from the surface of the membrane and the fibrin coagulates beneath it, separating it from the connective tissue ground work. Occasionally the cell row is broken up and the epithelial elements are found scattered through the fibrin.

The connective tissue ground work and the membrane contain a greater or smaller number of leucocytes. Where the leucocytes are many the amount of fibrin is usually diminished, and where the number of leucocytes are excessive the process is a fibro-purulent or simple purulent pleuritis.

The leucocytes have migrated into the tissue from the engorged vessels, the contents of which contain an excessive number of white cells. With suitable stains (Gram) micro-organisms (diplococci, streptococci, etc.) often in large number will be found lying in the tissue and in the membrane. These are the exciting agents of the process.

3. Pericarditis Adhesiva.

Where two serous surfaces are opposed to one another as in the pericardium, it frequently happens that through the deposition of fibrin these surfaces become temporarily adherent and if the adherence persists for a sufficient length of time they become firmly united and must be forcibly torn apart or cut through with the knife. When this stage is reached the adherence is no longer due to the mere agglutination of the fibrinous deposits, but through the transformation of the fibrin into connective tissue, the surfaces have practically grown together. (Plate XVIII., Fig. 2.) Formalin, F. M., N. S. or Mallory's connective tissue stain, elastica stain, van Gieson's or the last two together.

The microscopic examination of a long standing fibrinous exudative pericarditis, or pleuritis, discloses a characteristic irregularity in the line between the deposit and the underlying tissue. This line is no longer comparatively straight as in the fresh exudative process, but presents a very irregular appearance due to projections of tissue invading the fibrin. The examination of these projections with high power show them to consist of granulation tissue made up of spindle and round cells. Where the invasion of the fibrin by the organizing tissue is fresh this consists of spindle cells which spring from the outermost layers of the connective tissue of the serosa, at the same time the fibrin contains a large number of leucocytes. Where the organization is more advanced the number of spindle cells (fibroblasts) increases, fine capillaries filled with blood appear and beside the spindle elements large migrating round cells and lymphocytes make their appearance.

The origin of the different cell elements which go to form granulation tissue is by no means clear. The principal reason for this is that certain cells which are derived from the fixed tissue cells develop migratory abilities (histogenetic

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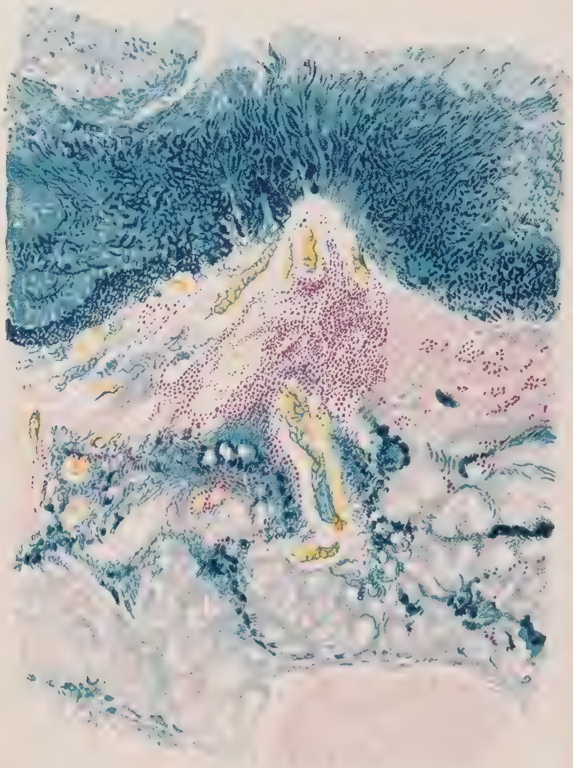
PLATE XVIII.

1.



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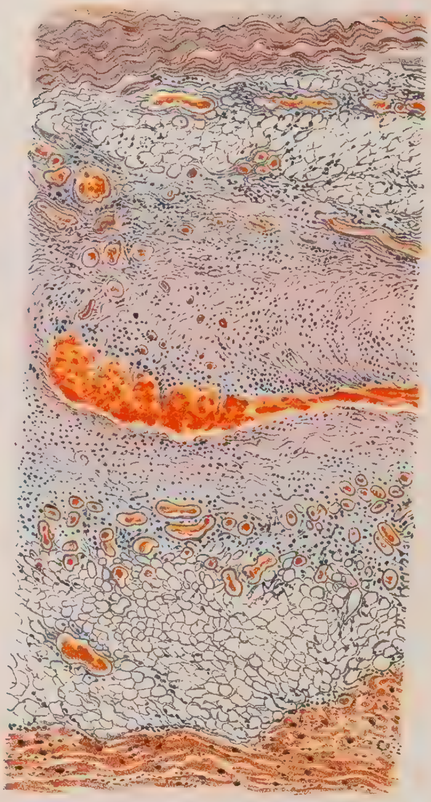

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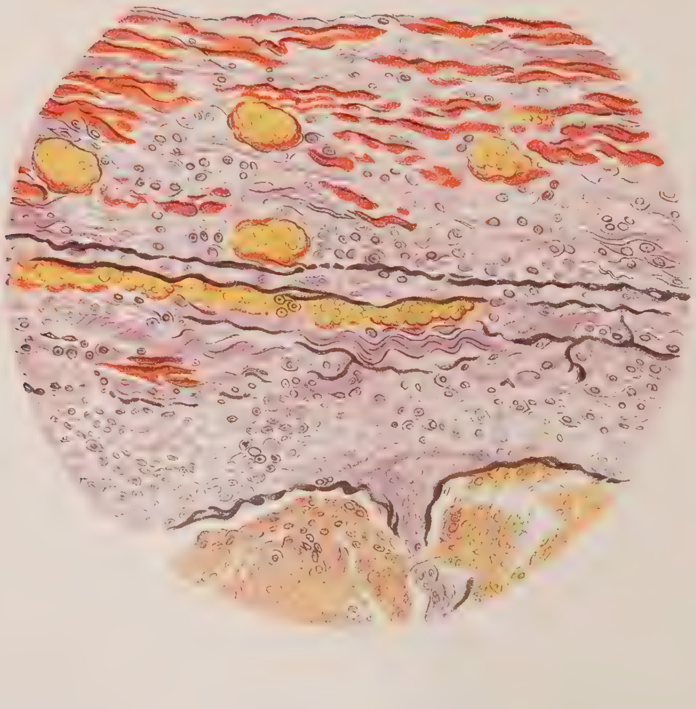

wandering cells) and become so altered that they appear very much as one type even when they are of different origin. Ordinarily one can distinguish three principal forms of cells in granulation tissue.

1. A predominating number of large spindle, at times more oval, and branching cells, with round or oval nuclei which possess a fine chromatin network. The cell body contains a good amount of protoplasm. These are derived from the fibrous connective tissue (fibroblasts) and later go to form the same.

A certain portion of the spindle elements are derived from the epithelium covering the serous membrane, the endothelium of the capillaries and the lymph spaces. These are credited with the ability to form fibrous connective tissue.

2. Large cells, for the most part round, with deeply staining protoplasm and round or slightly curved nuclei, very rich in chromatin, previously looked upon as large leucocytes which had migrated from the blood vessels. These are the so-called primary wandering cells of Saxer and are distributed through the connective tissue, but are found in greatest number about the vessels in the region of the adventitia (adventitia cells) from which they are supposed to spring. These cells likewise produce fibrous connective tissue.

3. Beside these forms a great number of small round lymphocyte-like cells, the third form of cell which goes to make up granulation tissue.

A portion of these take on an intense protoplasm stain (Unna's plasma cells). The great similarity which the larger forms and often the smaller forms of round cells show to the ordinary leucocytes precludes the possibility of definitely stating that a certain portion especially of the larger forms are not transmigrated leucocytes (hæmatogenous wandering cells). These hæmatogenous migrating cells, to which the polymorphonuclear leucocytes belong, never form connective tissue.

The formation of new capillaries occurs by the projection into the new tissue from a superficial capillary of a solid cord of endothelium. This later becomes tubular and the blood flowing into it transforms it into a capillary.

DESCRIPTION OF PLATE XVIII.

FIG. 1. Diphtheritic infection of the uvula. See p. 159. Low power. Carmine. Weigert's fibrin stain. Above a thick fibrinous pseudo-membrane, presenting a coarse-ribbed appearance (fibrin deep blue). At the surface the membrane sends fine projections into the submucosa. Marked accumulations of leucocytes in the submucosa. In the deeper layers of the tissue and in the engorged capillaries fibrin deposits. Below and to the right a bundle of smooth muscle fibers.

FIG. 2. Pericarditis adhesiva. See p. 176. Low power. Hæmatoxylin alizarin. Fibrin stained orange. Below the myocardium upon which rests the subpericardial fat tissue. Above this is a layer of newly-formed granulation tissue containing numerous capillaries. To the left this unites with a similar granulation tissue derived from the pericardial surface, the fibrous layer of which is seen at the extreme top of the picture. Below this a layer of subpericardial fat and upon this a mass of granulation tissue containing capillaries and showing marked fibrous transformation. At the center included between the two layers of granulation tissue is a mass of unorganized fibrin. This consists of hyaline clumps stained orange.

FIG. 3. Chronic tuberculous pleuritis. See p. 178. Hæmatoxylin, van Gieson, elastic fiber stain. Running through the tissue from left to right the basal elastic tissue layer of the pleura. All above this is newly formed connective tissue in which are included numerous bright red, hyaline, refractive bands. These are the remains of the fibrin which have undergone hyaline change. Upon the surface numerous enlarged capillaries, the walls of which show hyaline transformation. To the left and below a group of bright red fibers. At the base of the figure, two alveoli containing fibrinous exudate which unite through an opening in the interalveolar septum. In the fibrin, leucocytes and desquamated epithelium.

By this means the fibrin is gradually organized and transformed into connective tissue. The adherent fibrin layers serve as a convenient structure by which the granulation tissue projections from one side penetrate into the other and ultimately connective tissue and capillaries from both sides intermingle and all demarkation is obliterated. Occasional masses of hyaline-like material (altered fibrin) are included in the organized tissue, these usually lie at or near the boundary between the two surfaces and are remaining fragments of fibrin which have not been organized. (Plate XVIII., Fig. 2.)

To determine the original position of the serous surfaces the elastica in the section must be stained. (See next subject.)

4. Chronic Pleuritis.

All fibrinous deposits upon serous membranes when they persist for a sufficient length of time become transformed by a process of organization into fibrous connective tissue, when the roughened character of the surface disappears. The surface of the membrane is smooth and shiny, but on section is found to be distinctly thickened.

M. F., freezing microtome, N. S. or elastic stain, with or without van Gieson, formalin, or F. A., F. M., or embed. Fibrin stain.

Zenker. Embed in celloidin. Mallory's connective tissue stain.

To determine the relations of the various structures a section in which the elastic lamellæ are stained must be examined.

The position of the elastic layer shows us at once the original position of the surface of the membrane. The layer of elastic fibers is no longer as continuous as in the unaltered membrane, but is broken through at many points by capillaries and small vessels which penetrate into the connective tissue above. The derivation of these vessels from those of the serosa may be readily seen by the manner in which they penetrate the elastic layer.

If the organization has not been completed, we occasionally find bands of shiny hyaline material lying between the connective tissue fibers of the newly formed structure. These bands which stain yellow with picro-carmine, blue with Weigert's fibrin method, red with Mallory's connective tissue stain, red with van Gieson are altered fibrin which has undergone hyaline degeneration from long-continued isolation in the tissue. (Plate XVIII., Fig. 3.)

This statement is not meant to exclude a possible hyaline degeneration of connective tissue. Certain erroneous conclusions as to the nature of fibrin deposits have been drawn from the presence of these bands in advanced fibrinous membranes. It has been claimed that they represent the results of a peculiar degeneration of connective tissue into fibrin, and some observers have wished to attribute the entire fibrin deposit to this source.

Where the epithelium of the serous membrane is not totally desquamated, or does not take on fatty degeneration, certain portions of it may remain either beneath the membrane where in fresh cases karyokinetic figures representing an attempt at regeneration, may be observed, or the epithelium may grow up from the edges over the entire surface of the membrane. By this means the organized membrane receives a fresh covering of epithelium. Occasionally pockets and crevices are left between the fibrin deposit and the surface of the membrane, and here the epithelium which usually covers the side formed by the serosa, proliferates and lines the entire cavity, producing gland-like structures beneath the fibrin.

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These cavities and crevices, as well as the elastic layer, mark the position of the free surface of the membrane.

Such gland-like excavations are very common in the fallopian tube in extrauterine pregnancy, where one side of the tube is usually covered by an organizing deposit of fibrin. In the so-called milk patches of the pericardium these gland-like cavities are often found.

5. Tuberculous Pericarditis.

If tubercle bacilli find their way into a serous cavity (as in the pericardium through caseation of a neighboring lymph node) they cause, at least in the more acute cases, a fibrinous pericarditis or pleuritis. In these cases on stripping up the fibrinous membrane the rough and reddened surface of the pericardium is studded with minute gray dots, the specific indication of tuberculosis. In advanced cases where adhesions have occurred, masses of caseated material will be found.

F., F. M., N. S., or elastica stain. Tubercle bacilli stain. F., alcohol. F. M. or embed. Fibrin stain.

In sections made perpendicular to the surface, the character and extent of the membrane may be studied.

This is usually found to be thick and at the base in a state of advanced organization, masses of granulation tissue invading the fibrin. The granulation tissue on more careful examination does not correspond to the granulation tissue just studied, but shows a tendency to invade the fibrin in the form of isolated nodes in the middle of which caseation, and near the periphery of which giant cells appear. (Plate XIX., Fig. 4.)

Where the granulation tissue has not taken on the form of tubercles it usually invades the fibrin in a diffuse manner and shows a marked tendency to break down and caseate, and contains numerous typical tubercular giant cells. The tubercle bacillus, besides producing the fibrinous exudation, causes the development of tuberculous granulation tissue. In preparations where the elastica is stained the fibrin mass will again be found to lie above the elastic layer showing the greater part to be the result of exudation. The bacilli can readily find their way into the lymph channels of the serosa and thus produce tubercle formation in the deeper structures.

The hyaline bands mentioned under the last subject are especially numerous in tuberculous affections of the serous membranes. As these are occasionally found beneath the elastic layer it is impossible to exclude the possibility of their being derived from the connective tissue by a process of infiltration. (Plate XVIII., Fig. 3.)

Owing to the severe injury done the tissue by the soluble products of the bacillus, the connective tissue fibrils probably become permeated with the fibrin fluid and then undergo hyaline transformation. This is, however, not a direct degeneration of the connective tissue into fibrin.

IX. THE VASCULAR SYSTEM.

1. Endocarditis Verrucosa.

In certain diseases, especially inflammatory rheumatism, deposits are formed especially upon the leaflets of the mitral and aortic valves. These usually develop at the line of closure of the valves in the form of fine papillary eleva-

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tions which when fresh are readily displaced. If these fresh excrescences be examined after teasing in salt solution, they will be found to consist of fine granules, which on the addition of acetic acid disappear, showing them to be of albuminous character.

These are the so-called blood platelets, the third corpuscular element of the blood. They play an important rôle in the formation of thrombi and will be more carefully considered under that heading. Fresh excrescences upon the heart valves are produced by the deposition of these elements from the blood, and are usually found at the line where the valves close because at this point for at least a portion of the heart cycle they are held in place. Small injuries to the valve coverings are likewise frequent at the line of closure and these predispose to the deposition of the blood platelets.

F., or F. A., F. M., or embed. Fibrin stain.

M. F., F. M., or embed. N. S., or elastica stain.

Sections made perpendicular to the surface through such a deposit, show on examination with low power, the poorly stained mass sharply delimited from the tissue of the valve.

The surface of the deposit is bare, the endothelium stopping at the base.

By high power the endothelium covering the valve shows enlarged nuclei and swollen and deeply staining protoplasm.

At the point of attachment of the mass to the valve no marked alteration of structure may be noted, the endothelium is absent, and the nuclei of the connective tissue stroma are somewhat enlarged.

It is difficult in each case to determine exactly the conditions which have predisposed to the formation of the deposit. In the simple cases which do not persist, organisms are seldom found, although it is probable that organisms are the active agents of the process. Alteration and injury of the endothelium at certain points probably determine the site of the deposits. In later stages the deposit upon the valve leaflet changes in character and becomes firmly adherent. The mass is now more rounded or oval, is attached to the valve by a more or less well-defined pedicle and may be readily moved from side to side without becoming detached.

Perpendicular sections through the now well-formed wart-like projection show it to consist of fibrous connective tissue, very poor in nuclei. At the base and in the pedicle the tissue contains a greater number of nuclei and bands of spindle cells may be seen penetrating into the verruca in radiating form. (Plate XIX., Fig. 5.) The outermost portion of the wart consists of poorly stained granular and fibrous material, the remains of the blood platelets.

The original deposit of blood platelets is therefore being organized from the base and the process is that of organization of a blood platelet thrombus (see Thrombus), which is ultimately transformed into a mass of connective tissue (endocarditis verrucosa).

With the growth of the granulation tissue new vessels are formed. These spring from the vessels of the valve and penetrate to the base of the thrombus, through portions of the valves which normally do not contain vessels. Vessels thus formed usually remain and where the process is fresh the presence of vessels indicates the persistence of a previous process (endocarditis verrucosa recurrens). Deposits of pigment may be found in the valve tissue near the base of the verruca. These deposits are the result of hemorrhages from the newly formed vessels which are frequently injured by the opening and closing of the valves.

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PLATE XIX.

Fig. 1.

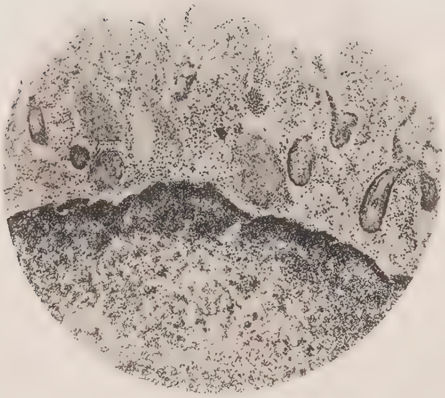


Fig. 2.

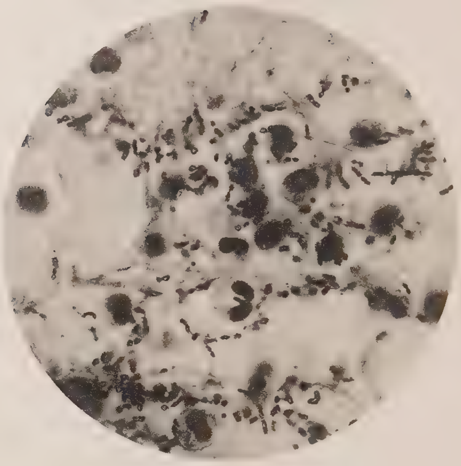


Fig. 3.

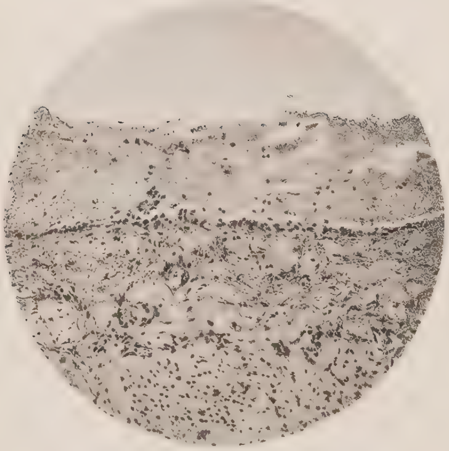


Fig. 4.

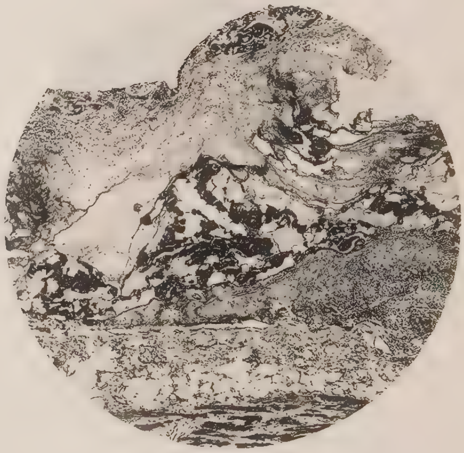


Fig. 5.

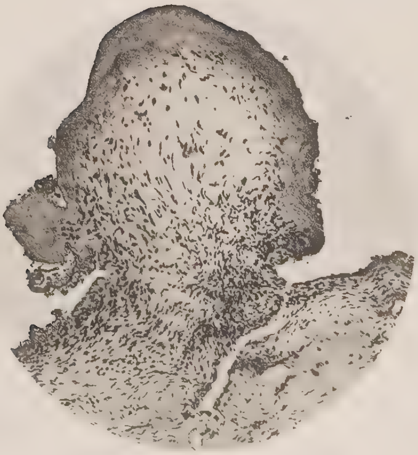
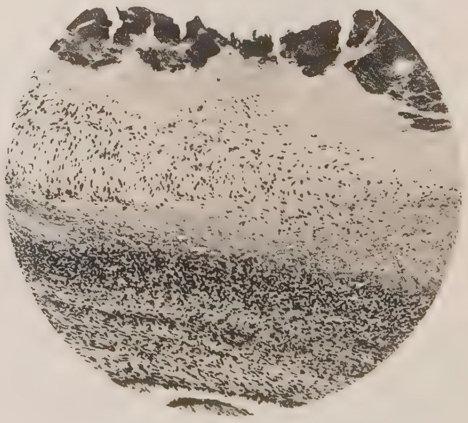


Fig. 6.



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2. Endocarditis Ulcerosa.

Beside the above-mentioned simple form of endocarditis, a second, the so-called malignant endocarditis, occurs.

The malignant form very commonly leads to, or is associated with, extensive metastatic abscess formation through the entire system. The valve leaflets present varied but characteristic lesions. Commonly the line of closure is covered with polypi of varying size, and the surrounding valve is thickened or ulcerated, and occasionally perforated. A peculiar worm-eaten appearance of the valve is common (endocarditis ulcerosa). A stained cover-glass preparation (Gram or methylene-blue) reveals the presence of large numbers of micro-organisms, usually cocci.

A section of an ulcerated valve (Plate XIX., Fig. 6) examined under low power, shows numerous deep blue masses upon the surface of the ulcerated portions, beneath this a zone lighter in color within which no nuclei are visible, and beyond this an area of deeply stained cell elements.

By high power the constituents of these zones may be determined. The deep blue masses upon the surface are found to consist entirely of cocci, which by the elaboration of toxines have produced a widespread total necrosis of the adjoining tissue, which is the colorless middle zone.

The deeply stained zone beneath the necrotic tissue owes its color to a dense infiltration of the tissue by leucocytes and round cells (zone of tissue reaction).

M. F., F. M. Gram.

DESCRIPTION OF PLATE XIX.

Fig. 1. Anthrax of the intestine. See p. 170. Low power. Carmine. Gram. Œdema of the mucosa and submucosa. Marked leucocytic infiltration, especially of the submucosa. Between the leucocytes a large number of fine rods (*magnifier*). These are closely packed in the upper layers of the submucosa and are the invading organisms, anthrax bacilli.

Fig. 2. Anthrax of the intestine. See p. 170. Carmine. Gram. Field from the submucosa of Fig. 1. Oil immersion. The œdema of the tissue is apparent and the field includes numerous leucocytes with curved nuclei. Between the leucocytes are longer and shorter rods, bacilli. Near the center a leucocyte with a fragment of a bacillus in its protoplasm (phagocytosis).

Fig. 3. Fresh fibrinous pericarditis. See p. 176. Middle power. Hæmatoxylin. Above and below a fibrin deposit which rests upon a continuous layer of epithelium which covers the surface of the pericardium (*magnifier*). In the superficial layers of the pericardial connective tissue a well-defined hemorrhagic infiltration (*magnifier*).

Fig. 4. Tuberculous fibrinous pericarditis. See p. 179. Low power. Hæmatoxylin alizarin. Below heart muscle. Above this pericardial fat tissue. Above this and to the right is a narrow cleft which separates the extensive fibrinous mass from the surface of the pericardium. In this cleft may be seen the remains of the pericardial epithelium (*magnifier*). To the right where the cleft ends is a nodular structure which unites the fibrinous deposit with the pericardium. This extends in the deeper layers of the membrane to the left and is composed of granulation tissue (*magnifier*) containing giant cells. The deeply stained portions of the membrane have undergone hyaline transformation. The fibrinous deposit at the uppermost surface is thickly infiltrated with cells (*magnifier*).

Fig. 5. Endocarditis verrucosa recurrens. See p. 180. Middle power. Hæmatoxylin. Below the section of a heart valve upon which rests a nodular projection (thrombus). At the base this is invaded by spindle cells which radiate into the mass (fibroblasts). In the valve structure numerous clefts and openings (vessels developed during previous inflammations).

Fig. 6. Endocarditis ulcerosa. See p. 181. Middle power. Hæmatoxylin. Cross section of a heart valve. Above deeply stained masses of organisms which have invaded the surface of the valve. Beneath these an unstained necrotic zone. Beneath this a dense round cell infiltration of the living tissue.

Toward the necrotic zone many of the leucocytes are broken down and chromatin detritus is common.

The organisms most commonly responsible for this necrosis of the valves are the streptococci. Occasionally the ordinary staphylococci of suppuration are the exciting cause and in rare cases the gonococcus has been found. Bacilli likewise have been observed and particularly the tubercle bacillus. In tuberculous endocarditis the verruche consist of thrombotic masses partly organized by tuberculous granulation tissue, in which typical giant cells and areas of caseation may be found. These thrombi contain masses of tubercle bacilli which when detached or disintegrated and carried into the circulation are capable of producing general acute miliary tuberculosis.

3. Arteriosclerosis.

Structure.—We distinguish three classes of blood vessels: arteries, veins and capillaries.

The capillaries are mere tubes of endothelium. The nuclei of the endothelial cells are oval in form, and the cells at their boundaries are held together by a form of cement. This cement substance may be stained with nitrate of silver when it appears as a black substance uniting the cells at their edges and filling in the interstices at the angles. The angles of the cells and where the cement substance is spread out are the points usually selected by transmigrating leucocytes, in passing through the capillary wall, and here occasionally minute openings are left (stomata).

The small blood vessels are distinguished from the capillaries by the possession of a coat of connective tissue surrounding the endothelial tube (adventitia). In the arteries as the small branches increase in size a third circular coat of muscle fibers, the media, makes its appearance between the endothelium and the adventitia, and in direct proportion to the size of the vessel these coats increase in thickness. In the definitely defined artery, a fourth structure, a layer of connective tissue, develops beneath the endothelium, separating it from the media. In the middle-sized vessels longitudinal bundles of muscle fibers are to be found in the adventitia.

The wall of the artery contains numerous elastic tissue fibers, which at the junction of the intima and media forms a definite layer, the membrana limitans interna, and at the junction of the media and adventitia a second layer, the membrana limitans externa.

In small and middle-sized arteries which are in a condition of marked contraction the elastica interna may be readily seen as a highly refractive convoluted line of elastic fibers surrounding the intima.

The number of the elastic lamellæ of the muscular (median) layer in the small vessels is not great, but in the large vessels (aorta, pulmonary vessels, carotids) they form a very considerable amount of the media and often obscure the characteristic appearance of the muscle fibers. The elastica externa is not so definite in the large vessels and the adventitia possesses no longitudinal bands of muscle.

The adventitia carries the vaso vasorum, the nutrient vessels of the artery. These minute branches in the larger vessels penetrate into the media but never reach as far as the intima.

The veins differ from the arteries, first, by possessing a more poorly developed media, which, in the smaller veins, consists of scattered circular bundles of muscle, supported by a relatively large amount of elastic fibers. The muscular constituents of the media are best developed in the veins of the lower extremities; in other regions, as the veins of the pia and dura, they are entirely absent. The adventitia of the smaller and middle-sized veins includes a considerable number of longitudinal non-striated muscle fibers.

Very rarely a few longitudinal fibers have been noted in the intima. The small arteries are distinguished by the prominence of the media, in which are relatively few elastic fibers, and the characteristic well-defined elastica interna; the small veins, by the small amount of muscle fibers and relatively great amount of circular

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elastic fibers in the media, and the lymph vessels by certain deviations from both of these forms, which we shall now consider.

The endothelium of the lymph vessels rests directly upon a loose layer of longitudinal and oblique muscle fibers, which are surrounded by a more or less distinct circular coat of muscle. The elastica of the lymph vessels does not form, as in the vein, a circular coat, but surrounds the muscle fibers and bundles with a fine network, similar to the distribution of the elastica in the media of the smaller arteries. The outer elastic layer of the lymph vessels is not sharply defined from the structures which surround the lymph vessel. In the larger lymph vessels more definite and larger elastic lamellæ appear, and these form an inner and outer delimiting elastic membrane.

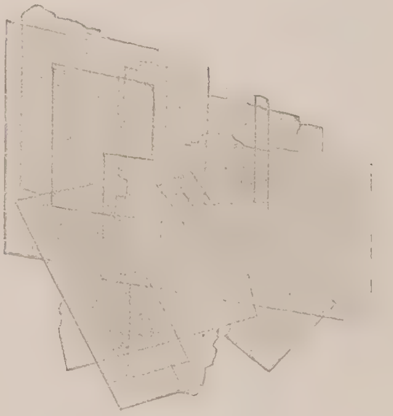
In advanced age the arteries usually undergo certain changes, which consist primarily of thickening and hardening of the arterial coats. This condition is known as arteriosclerosis. In the larger vessels, especially the aorta, this thickening of the coats is not uniform but appears in plaques and irregular patches (endarteritis nodosa). These patches are very irregular in shape, very commonly elevated and may be of transparent grayish white color with rounded edges, or in the larger patches, especially in the center, they present a distinct yellowish appearance. The smaller patches are usually whitish and firm to pressure; the larger patches are very commonly broken down at the center and present the appearance of excavated ulcers. The edges of these ulcerated patches are usually elevated and very hard, as if calcified. From the centers of the ulcers a shiny, smeary mass may be removed (atheroma). Certain arteries, as the arteria cruralis, may undergo a transformation into a stiff, hard tube (so-called pipe stem artery).

The examination of *fresh material* is best begun by picking out one of the small yellow patches or spots, and stripping up the intima with a fine pair of forceps.

The fine membrane thus removed is spread out on a slide and examined in potassium hydrate.

On first examination with low power the transparent membrane is found dotted with minute, black specks, which under higher power are resolved into fat droplets grouped together in the endothelial cells or the connective tissue fibrillæ of the intima. This fatty degeneration of the intima likewise occurs in the vessels of patients suffering from pulmonary tuberculosis, and is a simple passive degeneration of the cells.

Fig. 24.



Cholesterine crystals.

The examination of the degenerated mass from the ulcerated patches shows a large number of free fat drops and certain spherical bodies containing fat droplets. These bodies present in the center a clear space, which proves to be the nucleus of a cell, the protoplasm of which has taken up the numerous minute fat droplets (so-called fat droplet cell). These cells are either tissue cells or migrating cells. Beside these forms large semi-transparent rhombic or quadrilateral plates are present, from the corners of which rectangular pieces are commonly missing (Fig. 24). These are cholesterine crystals and give to the mass the shiny, pearly appearance at first noted. When treated with a solution of iodine and a few drops of sulphuric acid is added, the crystals, especially at the edges, take on a deep blue color.

The hardened character of the edges and floor of the ulcerated areas is due to calcification (deposition of calcium carbonate in the tissue). If a small piece of calcified artery be broken up and mashed under a cover-slip, and examined with high power the chalk in the arterial tissue may be seen in the form of fine, black granules. On the addition of acetic or hydrochloric acid a certain number of these granules disappear with the production of gas bells; the fat drops, however, remain.

The microscopic investigation of the changes in the arterial coats is best made on fresh material by the aid of double knife or freezing microtome sections, or hardened, when necessary decalcified and embedded, material, stained with N. S., van Gieson, elastica stain, polychrome methylene-blue, Mallory connective tissue stain.

Perpendicular sections of the arterial wall show that the media is most affected. The connective tissue fibers in this region are increased in number, enlarged and thickened and present a highly refractive hyaline appearance. The media as a whole is greatly thickened. Where the process is recent the nuclei of the connective tissue cells of the media are readily discernible and often increased in number. This is especially apparent at the point where the thickened patch joins the unaltered vessel wall. If the section be from a yellow patch, and fresh, or from tissue hardened in formaline, cut on the freezing microtome and examined in glycerine, a marked fatty degeneration of the intima cells and general fine granulation of the connective tissue fibers will be observed. The granules in the connective tissue fibers clear on the addition of potassium hydrate, the fat cells remaining.

In patches where the process is advanced areas of tissue which are totally necrotic are found and in these the elastic and connective tissue fibers are transformed into masses of detritus. The necrotic process begins in the deeper layers of the intima and extends to the surface, thus producing the excavated patches of ulceration.

Preparations in which the elastic fibers are stained show that these elements in the intima are destroyed and that the process usually halts at the membrana elastica interna. (Plate XX., Fig. 4.)

Occasionally the process extends deeper, invading the muscular coat, or isolated areas of necrosis may be found in the media, in which case other pathological alterations of the media are present. Where the media is affected we find accumulations and rows of cells infiltrating the muscular coat. The rows of cells usually surround small vessels which penetrate the media from without and spring from the adventitia.

These small vessels examined by high power are seldom found to reach the intima. The process is of inflammatory nature, affecting the media and associated with the formation of new vessels. Where areas of necrosis are present these are surrounded by the round cell infiltration and new vessels (mesarteritis).

In the necrotic areas the elastic fiber network of the media is destroyed. Besides the proliferation and necrotic areas more or less widespread deposits of chalk granules between the muscle fibers are to be noted. In sections which have not been completely decalcified these chalk granules take on a deep blue when stained with hæmatoxylon or Weigert's fibrin stain. (Plate XXI., Fig. 1.) Where the chalk deposit is not intense the muscle fibers retain their staining qualities; the elastica, however, is commonly broken up. In extensive areas of chalk deposit the muscle fibers likewise are destroyed. Where the sclerotic process is marked in the other coats, a distinct round-celled infiltration may be found in the adventitia surrounding the vaso vasorum.

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Arteriosclerosis is an inflammatory productive and degenerative process affecting the intima and media. This is associated with hyaline and fatty degeneration, necrosis and calcification of the tissue elements and cellular infiltration of the adjacent tissue.

According to Thoma the proliferation of the intima is preceded by degenerative changes in the media.

The exciting cause of arteriosclerosis is not definitely known; the most common factor in its production is advanced age. High arterial tension due to excessive muscular exercise, intoxication with alcohol, lead, and bacterial toxines are likewise probable factors in its production.

Syphilis is commonly held responsible for many cases of arteriosclerosis and in rare cases genuine syphilitic gummata have been found in the adventitia and media. Straub has recently described a form of syphilitic aortitis, found in cases of dementia paralytica where the change extends down to and stops abruptly in the neighborhood of the renal arteries.

Where the muscular coat of an artery is affected the vessel wall is very liable to dilate, and where the degeneration is irregularly distributed the dilatation takes the form of a sac (aneurysm). The muscular layer in the wall of an aneurysmal sac is usually very thin or entirely absent, while the intima is greatly thickened and covered with thrombotic deposits.

In the small vessels the degenerative form of the process is not as marked, while the productive character is accentuated. We find the principal changes in the small vessels to consist of a great thickening of the intima at the expense of the lumen of the vessel. This thickening may be very irregular affecting one side of the vessel, or it may be concentric. Where excessive the vessel may be entirely closed (endarteritis obliterans).

By staining the elastica in sections of small arteries we can readily determine the position of the membrana elastica interna and demonstrate that the thickening of the vessel wall due to proliferation of the spindle cells of the intima. (Plate XX., Fig. 5.)

In advanced cases a newly formed network of elastic fibers appears in the proliferating intima.

Obliterative endarteritis commonly affects the vessels at the base of the brain, the coronary arteries, and the vessels of the kidney (producing arteriosclerotic contracted kidney). (Plate IX., Fig. 2.)

A special form of productive or obliterating endarteritis is found in vessels which are surrounded by tissue affected by active and persistent inflammation. The best illustration of this form of endarteritis is found in vessels which penetrate the walls of tubercular cavities. (Plate XIV., Fig. 3.)

A still further form of endarteritis and periarteritis is associated with syphilis. (Plate V., Fig. 1.) Here the veins and lymph vessels are more commonly affected than the arteries and the affected vessels are usually surrounded by syphilitic tissue (as in various skin lesions of syphilis).

The process in the veins or lymph vessels usually leads to an endophlebitis (Plate XX., Fig. 6), or lymphangitis obliterans. The media and adventitia of these vessels likewise show marked evidence of cell proliferation. The structure of the veins is such that the loosely arranged layers of the media and adventitia are readily obliterated and may no longer be distinguished from the

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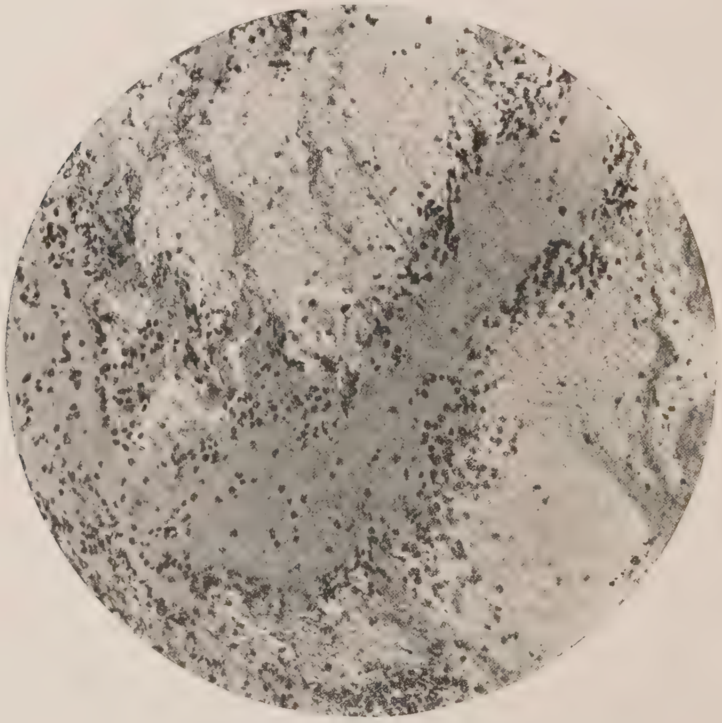
surrounding structures. For this reason it is always desirable to stain the elastica by which means the original boundaries of the vessels may be made out.

A productive endarteritis is found in tuberculosis, and consists of a proliferation of the intima which may extend into the media. The proliferative stage is followed by caseation and disintegration. Tubercles of the intima are most common in the pulmonary vessels, the small branches of the arteries in the kidney, and in the aorta. The walls of vessels are occasionally involved in the caseation spreading from adjacent tuberculous lymph nodes and in rare cases perforation follows.

4. Thrombosis.

The precipitation of the cellular elements of the blood (red and white corpuscles and blood platelets) in the living subject leads to the formation of a mass known as a coagulum or blood clot (thrombosis). The post-mortem blood clot differs from the true thrombus in many particulars. It is of one color, or consists of two layers, a white and a red; the surface is smooth and moist, it is of elastic consistence and is but loosely adherent to the vessel wall. A true ante-mortem thrombus is striated, being made up of irregular layers of red and white, the surface is covered with wave-like ridges, it is easily broken, is dry, and is more or less firmly attached at some point to the vessel wall. The fresh examination of the white portions of a true thrombus reveals an almost solid mass of blood platelets which are cleared on the addition of acetic acid.

Fig. 25.



Structure of a typical thrombus; the dark network is composed of blood platelets, at the margins deeply stained leucocytes; the light spaces contain red blood globules. (High dry power.)

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Rieder. Centralblatt f. allg. Path., etc., Bd. 9, 1898.
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PLATE XX.

Fig. 1.

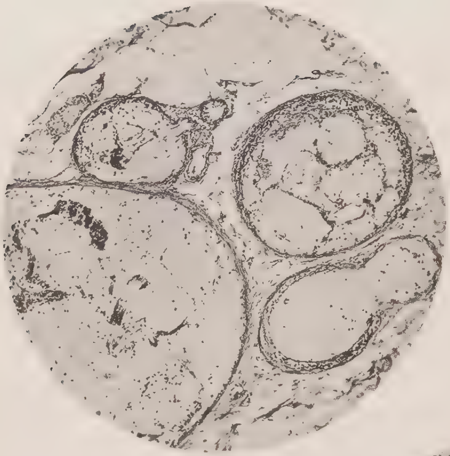


Fig. 2.

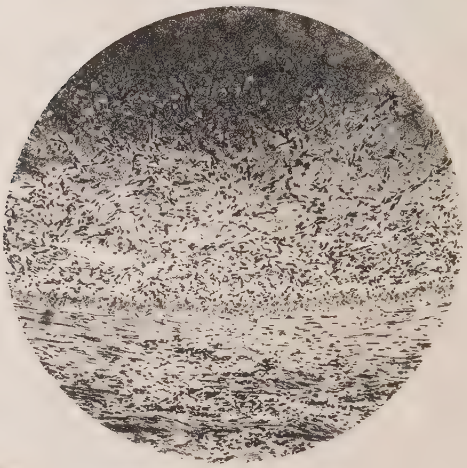


Fig. 3.

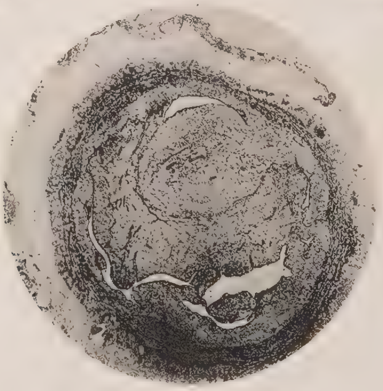


Fig. 4.

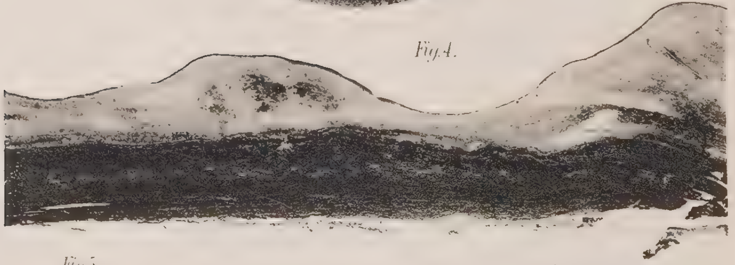


Fig. 5.

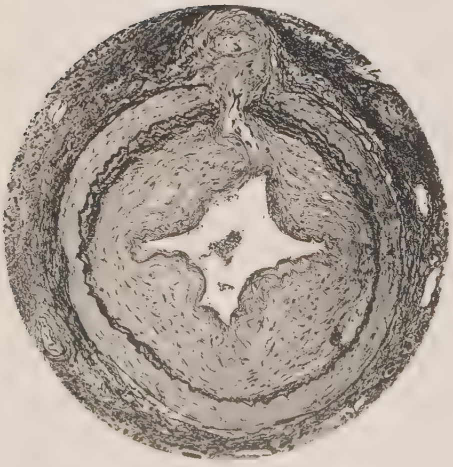
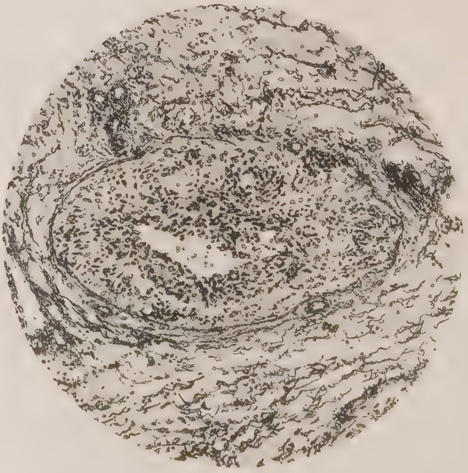


Fig. 6.



The finer structure of the thrombus can only be determined by the aid of thin sections.

F., F. M., embed. N. S.

F. A., embed. Fibrin stain.

By low power the characteristic structure of the thrombus becomes apparent and consists of a coarse net of unstained structure, the margins of which are deeply stained. The openings in this network are filled with red blood corpuscles. (Fig. 25.)

High power shows the network to consist of closely packed blood platelets. Toward the edges these are mixed with leucocytes which give the margins the stained appearance.

The first traces of fibrin may be detected among the leucocytes in sections stained with Weigert's fibrin method. The red blood cells in the interstices of the network show no pathological alteration. This relation of the various constituents of the thrombus leads us to the conclusion that the first step in the formation of the thrombus is the precipitation and agglutination of the blood platelets which form a sort of spongy or coral-like structure, through which the blood circulates.

Through the progressive precipitation of blood platelets the spongy network grows in bulk until it obstructs the blood stream and with the slowing of the current the leucocytes attach themselves to the margins of the structure. Thus

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DESCRIPTION OF PLATE XX.

FIG. 1. Peripheral distribution of leucocytes from slowing of the blood current. See p. 188. Low power. Hæmatoxylin. Engorged capillaries in the submucosa in cyanosis of the intestine. With *magnifying glass* the peripheral arrangement of the leucocytes may be observed. A certain number of leucocytes have likewise passed through the vessel walls and lie in the adjacent tissue.

FIG. 2. Organizing thrombus. See p. 188. Middle power. Hæmatoxylin. Below the muscularis of a vessel with long, slender nuclei (*magnifier*). Upon this the narrow intima from which springs a massive proliferation of granulation tissue composed of cells containing spindle-shaped and spherical nuclei. Above the remains of the thrombus which is invaded by large spindle cells with slender nuclei (fibroblasts) (*magnifier*). The muscularis of the vessel shows a marked round-celled infiltration.

FIG. 3. Fenestrated thrombus. See p. 189. Very low power. Hæmatoxylin. The original wall of the vessel is indicated by the deeply stained muscular coat. The original lumen of the vessel is filled with a mass of connective tissue, in which above and below may be seen more or less extensive openings and clefts. These are the newly-formed vessels which have penetrated the thrombus and through which the circulation has been partially restored.

FIG. 4. Atheroma of the aorta. See p. 184. Very low power (micro-collinear). Longitudinal section. Hæmatoxylin. Elastic fiber stain. The media with its rich elastic tissue is deeply stained. Upon this is the irregularly thickened intima. In the irregular projections of the intima may be seen deeply stained foci of degeneration.

FIG. 5. Endarteritis productiva. See p. 185. Middle power. Hæmatoxylin. Elastic fiber stain. Small artery from the kidney. The elastica interna with its wavy lamellæ is distinctly seen. Without this the muscularis of the vessel and surrounding this a well-defined elastica externa. Within the elastica interna the intima shows massive proliferation which has encroached upon the lumen and throws the proliferated tissue into folds. Above a small vasa afferentia and a hyaline glomerulus. The proliferation has occluded the lumen of this vessel (endarteritis obliterans).

FIG. 6. Endophlebitis productiva. See p. 185. Middle power. Hæmatoxylin. Elastic fiber stain. Small vein without well-defined muscular coat. Elastic fibers of the vessel wall well defined below and to the left. Above and to the right the elastic coat is broken. The intima shows massive proliferation and contains a large number of cells with spindle and oval nuclei.

the current flows more and more slowly until only a filtering through of serum remains, and then the formation of fibrin begins. This makes its appearance among the leucocytes in the form of fine threads, but soon develops into bands stretching from one rib of the lattice to the other, or surrounding the spongy network of blood platelets.

The formation of the fibrin is brought about by the elaboration of a fibrin ferment from the bodies of the leucocytes, among which the first beginnings are noted.

This ferment acting on the fibrin-forming element of the plasma (fibrinogen) produces the fibrin.

The formation of fibrin in a thrombus, is therefore, a purely secondary process, the result of the disintegration of the leucocytes.

In thrombi, which have existed for some time, the different elements may no longer be distinguished. The blood platelet structure, disintegrating leucocytes, and fibrin, gradually unite in the formation of hyaline masses, between which clefts and open spaces appear, giving the whole much the appearance of certain fibrinous pseudo-membranes. The remains of red blood corpuscles are found in the form of yellowish brown pigment within the thrombus mass.

Various conditions predispose to the formation of thrombi, the principal being any condition which causes slowing of the blood current. For this reason thrombosis most commonly affects the veins. In cross sections of veins in which stasis has occurred (in cyanosis of various organs or of the intestine) we find an unusual distribution of the blood cells. The leucocytes, although not present in unusual number, are arranged about the periphery of the vessels (Plate XX., Fig. 1), and here and there small masses of blood platelets may be seen. Leucocytes with elongated nuclei in the act of penetrating the vessel walls are likewise seen.

5. Organization of Thrombi.

The greater the age of a thrombus the more difficulty is experienced in removing it from its attachments to the vessel. On microscopic examination of sections of such thrombi (F., F. M., N. S.), this adhesion to the wall of the vessel is found to be due to a growth of cells from the intima into the substance of the thrombus. (Plate XX., Fig. 2.)

By examination of the section with high power we find this growth to consist of round and spindle cells which have invaded the substance of the thrombus. The spindle cells in particular penetrate between the hyaline masses of the thrombus. These cells spring from the endothelium or from the connective tissue cells of the intima and are the forerunners of complete organization. Here and there areas in which the organization is more advanced may be seen, and here newly formed capillaries appear. These spring from the vessels of the media and penetrate through the intima.

The cells of the intima often contain the characteristic hæmatogenous pigment which is formed from the hæmoglobin of the disintegrated red blood cells.

When the thrombus completely fills the lumen of the vessel the transformation of the mass into connective tissue may securely close the lumen of the

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vessel. Later, the circulation is occasionally reëstablished by perforation of the connective tissue occluding the lumen.

The method by which this is brought about is best understood by the examination of an old organized thrombus, where the connective tissue of the thrombus mass is penetrated by large capillaries. These enter the thrombus not only from the wall of the vessel, but from the free surfaces of the thrombus, in which case they are lined by endothelium which is continuous with that of the patulous lumen of the vessel, which proliferates upon the surface of the thrombus and penetrates the mass in the form of capillaries. These capillaries carry blood directly from the lumen of the vessel and under the influence of the blood pressure dilate and penetrate deeper and deeper into the connective tissue until anastomosing with capillaries from the other aspect of the thrombus, a communication is established. This capillary communication gradually widens, and by this means the blood current may be to a greater or less extent reëstablished (canalized thrombus). (Plate XX., Fig. 3.) About the newly formed capillaries is formed a distinct network of elastic fibers.

6. Endophlebitis Septica.

In large thrombi, especially in the cavities of the heart, the center of the thrombus is free from fibrin formation, giving the center of the mass a less consistence than the surface.

The leucocytes and blood platelets in this region, on disintegration, leave a granular, mushy material (softened aseptic thrombus). This central softening of an aseptic thrombus has nothing in common with the softening which occurs in thrombi containing micro-organisms (septic thrombus). As a result of the action of these organisms the thrombus breaks down into a discolored smeary mass (puriform softening). *A stained cover-glass preparation* serves for the detection of organisms in large numbers. Septic thrombi are formed most readily in veins which draw their supply from the neighborhood of infected wounds. If such a vein be dissected out, a discolored appearance of the wall of the vessel is found, at the site of the thrombus.

F., F. M., N. S., or Gram or elastic fiber stain.

The greater portion of the thrombus is found on examination with high power to contain large numbers of micro-organisms. The remains of leucocytes in the form of granules and fine masses of chromatin, mark the site of their destruction. The protoplasm of the leucocytes and the blood platelets disintegrate into a fine granular material which does not stain readily. The wall of the vessel shows marked areas of necrosis in the neighborhood of which masses of bacteria are found. The portions of the vessel wall which are not necrotic are in a marked state of reaction, being permeated by polymorphonuclear leucocytes and round cells. If the bacteria perish, the thrombus mass is organized and a plug of scar tissue usually marks the site of the lesion.

X. THE LYMPH NODES.

Structure.—The lymph nodes are small, bean-shaped structures into the convex side of which the afferent lymph vessels enter. These spread out through the substance of the node in the form of channels or sinuses and converge at the concave side or hilum of the node, emerging again as vessels, the vasa efferentia. The spaces in the node

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between the lymph channels are filled with lymphocytes, which are found in radiating groups at the medulla of the gland and in the cortex form into more or less spherical groups, the follicles. The follicles and radiating structures of lymphocytes in the medulla come into direct contact with the lymph stream. A specially definite lymph channel directly beneath the capsule of the node bears the name of peripheral sinus. The centers of the follicles often appear less deeply stained than the periphery, and here large cells often in the act of dividing will be found. These are the so-called centers of proliferation (Flemming). The entire node is divided and supported by a connective tissue structure which is continuous with the capsule, and which, accompanied by the lymph channels, surrounds and penetrates between the follicles. In many places the finer connective tissue structures unite in a larger structure, a trabeculus. The arteries of the lymph node run in the long axes of the radiating masses of lymphocytes in the medulla, and leave the node at the periphery.

1. Acute Lymphadenitis.

Following the absorption of toxic substances into the body, as from the intestinal canal, the lymph nodes in the neighborhood of the point of entry of the toxines, become markedly swollen and on section contain more fluid than normal.

M. F., F. M., N. S.

The microscopic examination of the section shows at once a marked widening or dilatation of the lymph channels within which are an unusual number of cells. In the normal node but few leucocytes appear in the lymph sinuses and these of the mononuclear variety. Here the greatly increased number of cells in the lymph channels consist, besides lymphocytes, of a large number of polymorphonuclear leucocytes, and larger elements with multiple nuclei and bodies containing a large amount of protoplasm. These last named cells closely resemble epithelium, and in fact are the desquamated endothelial cells which cover the trabeculæ of the node and line the lymph channels (so-called lymph sinus catarrh).

If the toxic substances are capable of producing fibrinous exudation, as in fibrinous pneumonia, where the tracheal nodes are affected, the lymph channels are often plugged with fibrin beside containing red blood corpuscles, coal dust from the lung, and the usual leucocytes and desquamated endothelium.

2. Purulent Lymphadenitis.

If pus-forming organisms enter the lymph channels and are carried to the lymph nodes, circumscribed collections of pus cells, or abscesses, are formed. The best example of this form of invasion of the lymph nodes are the buboes which frequently accompany chancroids.

M. F., F. M., N. S., or methylene-blue.

The demonstration of the specific bacilli of chancroid is difficult of accomplishment, so that, as a rule, only the histological changes need be noted.

By examination of the section with low power we find the typical structure of the node is obscured by an extensive accumulation of cells. The centers of these cell masses are usually lost in frozen sections, so that it is common to find a large hole in the section at what should be the center of the abscess.

By high power the cells are found to be polynuclear leucocytes of the type which we recognize as pus cells.

The lymphoid elements are practically obscured.

The vessels of the capsule are markedly engorged, and the lymph sinuses, as a result of the marked increase in the elements within the capsule, are obliterated.

Purulent inflammation, with the formation of true abscesses and dissolution of the tissue, constitutes the process.

3. Inflammatory Necrosis of the Lymph Nodes.

When necrosis-producing organisms instead of the pus-forming varieties invade the lymph channels and nodes the picture is somewhat altered.

The best example is found in the mesenteric lymph nodes in typhoid fever, when the typhoid bacilli are carried into the nodes. On section the enlarged pulp protrudes above the surface, and presents a whitish appearance with here and there an opaque, yellow spot.

M. F., F. M. Methylene-blue.

F., or F. A. Embed. Fibrin stain.

In sections the enlargement of the nodes is found to be due to a great increase in the number of lymphocytes. The yellow spots are represented in the sections by unstained necrotic areas, and in favorable sections even with low power deep blue masses will be found, which, under high power, are resolved into clumps of slender blue bacilli, typhoid bacilli. The necrotic areas contain a marked amount of fibrin, as may be seen on staining the section with Weigert's fibrin method.

A similar necrosis of the lymph nodes of the neck occurs in severe cases of diphtheria. Through the marked increase of the lymphocytes and the invasion of the node with leucocytes from without, the boundaries between the follicles and lymph channels become obliterated. In the follicles unstained areas appear which must not be confused with the centers of proliferation; these on examination with high power are seen to be due to necrosis of the tissue. Within the necrotic areas are found the remains of cells, chromatin granules and detritus embedded in masses of fibrin, the whole presenting a picture very like the conditions found in pseudo-membranes. The amount of fibrin may be determined by appropriate staining and is found to be great in the necrotic areas, the lymph channels likewise containing a considerable amount. Very extensive areas of necrosis are occasionally observed. The process consists of necrosis of and exudation into the parenchyma of the lymph node produced by the presence of the diphtheria bacillus, or by the action of its toxines. The organisms may occasionally be stained in the proliferating portions of the parenchyma. (Loeffler's methylene-blue, Weigert's fibrin method.)

4. Tuberculosis of the Lymph Nodes.

The tubercle bacillus produces two forms of alteration of the lymph nodes. In the first the node contains minute gray dots, the centers of which may be caseated; in the second the node is markedly enlarged, and on section presents an appearance not unlike a boiled potato. This peculiar appearance of the cut surface is produced by diffuse caseation of the node (scrofula).

M. F., F. M., N. S.

In sections from the first form of tuberculous infection the gray dots are found to be typical epitheloidal tubercles, usually located near the centers of the follicles. As the centers of cell proliferation (Flemming's) occupy a similar position in the follicles and as at these points large proliferating cells are found, it is not always easy to distinguish between a center of cell proliferation and a beginning tubercle. The presence of tubercle bacilli, giant cells, and caseation will determine the diagnosis in tuberculosis. In chronic cases the tubercles may be more fibrous in character with beginning hyaline degeneration, which markedly alters the picture when the tubercles are fresh.

In scrofulous lymph nodes (Plate XXII., Fig. 2) the greater portion of the

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structure is necrotic. The edges of the necrotic areas are surrounded by granulation tissue, which by the absence of capillaries and the presence of epitheloidal and giant cells, within which the specific organisms may be stained, is recognized at once as tuberculous. Occasionally typical tubercles may be found near the margins of the necrosis. Where the tuberculous process is long standing the portions of the node which are not necrotic show evidences of proliferation in the cells of the reticulum. The tissue formed in these portions of the nodes is characterized by the large size of the newly formed cells. Occasionally the fibrous structure of the reticulum is most prominent, and hyaline degeneration of the reticulum is not uncommon.

5. Absorption of Blood Cells by the Lymphatics.

In widespread hemorrhage into the tissue, the adjacent lymph nodes appear markedly red, and on section the pulp has a red, mottled and striped appearance.

M. F., F. M., carmine or hæmatoxylin, eosin.

Under low power the lymph channels are seen to be enlarged and contain numerous yellow masses, which by high power are found to consist of well-preserved red blood globules. (Plate XXII., Fig. 1.) After being absorbed into the lymph spaces of the tissue the extravasated red blood cells have found their way into the nodes and lie in the lymph sinuses. Between the red blood cells are found the lymphocytes common to the lymph beside certain large round cells with central round nuclei and distended cell bodies in the protoplasm of which are well-preserved red blood cells (phagocytic cells). (Plate XXI., Fig. 2.)

To what extent these cells are endothelial in origin or derived from the lymphoid elements it is difficult to say. At least the cells of the parenchyma adjacent to the lymph channels and the endothelium lining them contain traces of the process in the form of coarse platelets of hæmatogenous pigment.

Whether the pigment in the cells indicates that these cells formerly contained red blood globules which have disintegrated within the cell, or whether the pigment has been taken up by the cells after the disintegration of the free red globules in the lymph channels may not be determined.

DESCRIPTION OF PLATE XXI.

Fig. 1. Arteriosclerosis. See p. 184. Low power. Hæmatoxylin. The deeply-stained mass at the center of the muscular coat is chalk (stained blue with hæmatoxylin). Above the elastica interna. Below the adventitia of the vessel (longitudinal section). The intima shows a moderate amount of proliferation.

Fig. 2. Phagocytic cells from the spleen in typhoid fever. See p. 192. High power. Hæmatoxylin. A group of large cells with deeply stained oval nuclei which contain in their protoplasm numerous well-preserved red blood corpuscles.

Fig. 3. Anthracosis of a lymph node (early stage). See p. 193. Low power. Carmine. Rich deposits of coal dust in the lymph sinuses. The follicles of the lymph node are practically free from deposit.

Fig. 4. Anthracosis of a lymph node (advanced stage). See p. 193. High power. Hyaline fibrous connective tissue. The coal dust is deposited between and in the bodies of the slender spindle-shaped connective tissue cells.

Fig. 5. Deposits of hæmatogenous pigment in the spleen in typhoid fever. See p. 196. Pigment consists of coarse brown granules accumulated in groups. The stroma of the spleen is greatly thickened.

Fig. 6. Mast cells. See p. 70. High power. Unna's polychrome-blue method. (From a myoma of the uterus.) Large cells with blue nuclei. Protoplasm filled with coarse, bright red granules.

Fig. 7. Plasma cells. See p. 177. High power. Unna's polychrome-methylene-blue method. The protoplasm of the cells shows a coarse deep blue granulation. (The nuclei in the figure are shown somewhat too dark.)

PLATE XXI.

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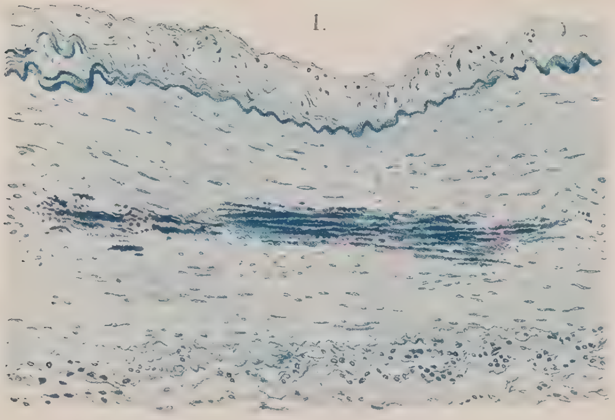

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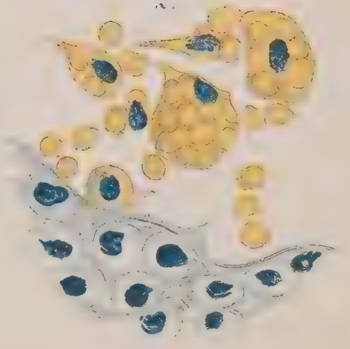

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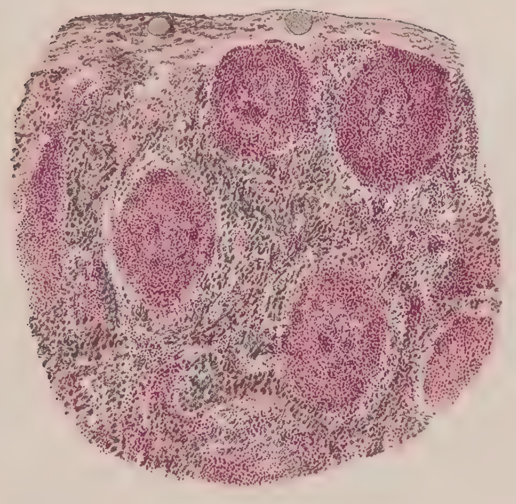

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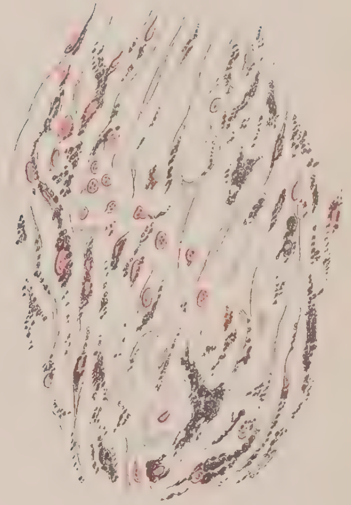

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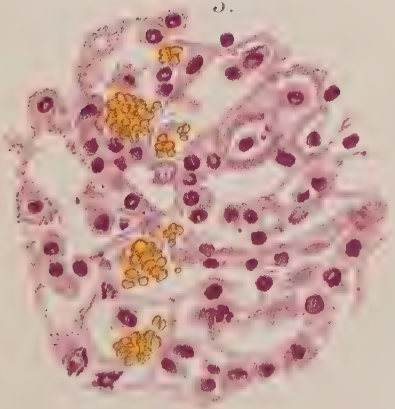

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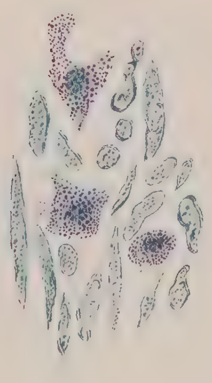

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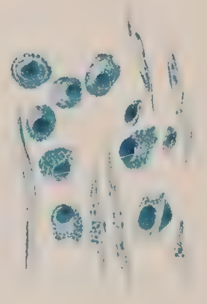

It is of course possible that both processes may contribute to the deposits of pigment in the cells lining the lymph channels and the adjacent cells of the parenchyma.

6. Anthracosis.

In exactly the same manner as the red blood cells, insoluble substances which find their way into the tissue are carried by the lymph channels to the nodes and there deposited. The substance most commonly deposited in this manner is the coal dust which enters the lungs on inspiration and then finds its way through the lymph channels into the bronchial lymph nodes. The deposit of coal dust in these nodes is often sufficient to give them an intense black appearance, and on section the structure of the node is no longer apparent, the incision producing a smooth black surface (slaty induration). The elasticity of the structure diminishes as the process advances, the node becoming progressively denser.

M. F., F. M., carmine.

The microscopic appearance of a lymph node in which the deposition of coal dust is beginning, presents an appearance very like that following the absorption of blood cells, except that instead of the brownish yellow pigment, the cells lining and adjacent to the lymph channels contain opaque black granules of varying size. (Plate XXI., Fig. 3.) By high power we find that the coal dust is deposited in the protoplasm of the cells, particularly the endothelium, where the granules are often arranged in a spindle-shaped mass, in the center of which are embedded the red nuclei of the cells.

The continued deposition of the coal dust ultimately produces irritation of the tissue elements, which is followed by proliferation of the endothelium of the lymph channels and the connective tissue cells of the stroma.

By this means the microscopic appearance of the node is completely changed. Instead of lymph channels and follicles, the node consists of dense fibrous connective tissue arranged in concentric layers.

Between the layers of the fibrous tissue we find the coal dust arranged in spindle-shaped masses, showing that it is deposited in the protoplasm of the younger connective tissue elements. (Plate XXI., Fig. 4.) The connective tissue is very commonly affected by hyaline degeneration and the center of the mass may become necrotic, in which case the disintegration of the cells liberates the coal dust, which is disseminated in clouds through the necrotic material.

7. Metastatic Deposits of Carcinoma in the Lymph Nodes.

A peculiar quality of all malignant epithelial tumors is that they early affect the neighboring lymph nodes, producing in them secondary deposits like the original tumor. The beginning of this change in the node may not be detected with the unaided eye. In carcinoma of the breast the axillary lymph nodes commonly afford good examples of the beginning invasion.

M. F., F. M., N. S.

By low power we find that the peripheral sinus of the node is filled with cells while the other lymph channels are unaltered.

By high power these are found to be large, closely packed masses of epithelial cells, identical in appearance and staining qualities with the cells of the carcinomatous growth in the breast. Where the character of the original tumor is more adenomatous, the cells of the metastases in the lymph nodes are large cylindrical elements, arranged side by side.

This massing together of epithelial cells, or the formation in rows of cylindrical cells constitutes the characteristic difference between metastatic invasion of the node with carcinoma, and desquamated endothelium found in the lymph channels during acute lymphadenitis. Beside the arrangement of the cells, the character of the nuclei, their richness in chromatin, and the deeply stained protoplasm of the cell bodies, serve to distinguish the epithelial cells of carcinoma from the desquamated endothelial elements, the oval nuclei of which contain but a small amount of chromatin.

XI. THE SPLEEN.

Structure.—The spleen possesses a connective tissue support, which spreads from the hylum in a coarse network (trabeculæ) toward the periphery and carries in its larger divisions, the branches of the splenic artery. This trabeculous network, which likewise contains a few non-striated muscle fibers, becomes continuous at the periphery with the capsule of the spleen. From the sides of the thicker trabeculæ spring minute connective tissue divisions between the meshes of which the pulp of the organ is supported. The pulp consists for the most part of large round mononuclear cells, in appearance identical with the mononuclear leucocytes of the blood (lymphocytes). Beside these elements the pulp commonly contains nucleated red blood cells, large phagocytic cells containing red blood globules, or blood pigment, and lastly large giant cells similar to the giant cells of bone marrow (see Bone Marrow). The smaller branches of the splenic artery are surrounded by an accumulation of lymphocytes which accompany the vessels to just before the point where they merge into capillaries. Cross sections of the arteries with the surrounding cells give the appearance of circular structures in the pulp (Malpighian corpuscles). The small arteries which have no connective tissue support (trabeculæ) are likewise surrounded by lymphoid tissue. Only when the last divisions of the arteries are reached, where the branches unite with the capillary system, is the lymphoid structure absent. The arterial capillaries are supposed to enter directly into the large veins of the spleen. These are lined with long sickle-shaped endothelial cells. When these elements are isolated the center of the cell body is found to swell outward about a large central nucleus. The loose structure of the spleen and the thinness of the vessel walls readily permit of the passage of foreign bodies or substances from the vessels into the pulp of the organ, where they are deposited in the crevices of the reticulum.

1. Splenic Infarction.

Occlusion of the branches of the splenic artery lead to the formation of infarcts in the spleen. The infarcted area is pale in appearance and rapidly becomes necrotic.

M. F., F. M., N. S.

The vessels in the infarcted area with the surrounding lymphoid tissue obtain a certain amount of nourishment from the occluded vessels, and therefore preserve their staining qualities after the surrounding tissue has become necrotic.

In old infarcts the entire tissue is necrotic, and the edges show beginning organization. In place of a hemorrhagic zone the boundary between dead and living tissue is marked by groups of yellow granular pigment and pigmented cells. Pigment in the form of hæmatoidin needles is occasionally found near the center of the infarct. If the vessel be occluded by an infected embolus, a septic infarct with a purulent demarkation zone is formed (see Septic Infarct of Kidney).

2. Acute Hyperplasia of the Spleen.

(*Acute Splenic Tumor.*)

In typhoid fever, acute malaria, recurrent fever and in many septic infections, the spleen is markedly enlarged. The capsule of the organ is tense, and on section the dark-red pulp rises over the cut surface and may be readily scraped away with the knife. The trabeculæ and Malpighian corpuscles are indistinct or invisible. If scrapings from the cut surface of the organ be examined in salt solution, various cell forms may be distinguished. First, small lymphocytes from the Malpighian corpuscles; second, large granular cells, the pulp cells which resemble the large mononuclear cells of the blood; third, polymorphonuclear leucocytes; fourth, narrow, sickle-shaped elements with protrusion of the protoplasm about the large central nucleus (endothelium of the splenic veins), and fifth, large distended elements, the protoplasm of which contains red blood globules, or fragments of the same. The presence of these last-named phagocytic cells indicates that a process similar to that in the lymph nodes following absorption of hemorrhage is in progress, and that certain of the fixed and migrating cells of the spleen are engaged in taking up the free red globules of the pulp. Compared with scrapings from the pulp of a normal spleen, the endothelial elements, and phagocytic cells are greater in number in the acutely enlarged organ.

Degenerative processes, especially fatty degeneration of all of the cell elements mentioned, are usually present. In typhoid enlargement of the spleen *cover-slip preparations stained with methylene-blue* disclose the presence in the pulp of the typhoid bacillus.

A somewhat more simple task is the detection of *malarial organisms* in the spleen, and the *spirillæ of recurrent fever*, both of which may be readily detected in *fresh preparations of the pulp.*

M. F., F. M., N. S. For finer sections embed in paraffine and fasten to the slide, or transfer to celloidin.

The examination with the microscope shows that the enlargement of the organ is due to increase in number of the cells constituting the pulp and to marked hyperæmia. The greatest increase is in the pulp cells, but the polymorphonuclear leucocytes are likewise more numerous than in the normal organ. Occasionally we find a cell which has taken up the red blood globules, which fill every crevice between the pulp cells.

In typhoid spleen small spots of necrosis occur, in which, in appropriately stained sections, the typhoid bacilli are found. Malarial organisms, and the spirillæ of recurrent fever may likewise be demonstrated in stained sections.

In diphtheria where the spleen is enlarged, this is the result of swelling of the pulp and Malpighian corpuscles, although the latter are more markedly enlarged than the pulp.

M. F., F. M., N. S.

By high power small areas of necrosis in the enlarged Malpighian bodies are to be noted. These are analogous to the necrotic foci in the lymph nodes in diphtheria.

3. Chronic Hyperplasia of the Spleen.

(*Chronic Splenic Tumor.*)

(*a*) Cyanotic spleen.

Chronic heart disease and certain chronic lung affections which lead to cyanosis of the abdominal organs produce marked alteration of the spleen. The organ becomes enlarged, the consistence increases, the capsule tense and often thickened, the cut surface smooth and of deep red color and the trabeculæ plainly visible. *A fresh scraping* shows the presence of a great number of red blood corpuscles.

M. F., F. M., N. S. or alcohol hardening, van Gieson. Zenker, Mallory connective tissue stain.

In long-standing cyanosis of the spleen the microscopic examination shows a marked thickening of the reticulum of the pulp. The engorged capillaries and veins stand out clearly. In the veins the protruding nuclei of the endothelium may be observed. Where the process is far advanced the cells of the pulp are decreased in number, the lymph follicles are small and the trabeculæ greatly thickened. The connective tissue structure and pulp cells contain granules of hæmatogenous pigment. The deposit of pigment is occasionally great. (Plate XXI., Fig. 5.)

(*b*) Following typhoid fever or septic infection the spleen gradually diminishes in size by the dissolution of the pulp cells which take on fatty degeneration, or are carried bodily from the spleen by the circulation. The remains of the process may be found in deposits of granular hæmatogenous pigment in the connective tissue cells about the small arteries and in the cells of the pulp, but never in the lymphoid elements of the follicles. (Plate XXI., Fig. 5.) (*M. F., F. M. Carmine.*)

Where the irritation of the spleen is long continued, or the acute enlargement recurs often, the organ remains permanently enlarged, but is not as hyperæmic and soft as in the acute swelling.

The microscopic examination (*M. F., F. M. Carmine*) shows the enlargement to be due to increase in the elements of the pulp and follicles. Alterations of the supporting structure of the organ are moderate, consisting of a slight thickening of the reticulum and trabeculæ, and deposition of hæmatogenous pigment.

In other forms of chronic enlargement of the spleen (chronic malaria) the thickening of the reticulum is so marked as to be visible to the unaided eye. The cut surface is brown, or in malaria presents a grayish surface with black spots and areas.

The follicles in some cases are very definite, in others absent, and in very severe cases the entire structure of the spleen is indistinct or lost.

M. F., F. M., N. S. Elastic fiber stain and van Gieson.

Microscopically the connective tissue is found to be greatly increased, especially in the pulp, where the reticulum has changed into thick fibers with definite nuclei. The number of capillaries is small, the lumena of the larger ones diminished. The cells of the pulp are only present in patches and are loaded with pigment, both hæmatogenous and black pigment (melanine) from the malarial parasites. This pigment is formed in the bodies of the developing parasites, is freed when the organism segments, and is distinguished chemically by being free from iron. The follicles may be enlarged, or may be reduced to a few accumulations of lymphocytes about the vessels.

(*c*) The macroscopically characteristic enlargement of the spleen in the newborn presents a variable microscopic structure. In some cases the pulp is very hyperæmic and hyperplastic, the follicles increased by a marked proliferation of large elements (hyperplastic centers of proliferation). About the vessels in the trabeculæ a diffuse, small, round cell infiltration is found. In hereditary syphilis gumma formation and proliferation of the connective tissue produce the enlargement of the spleen.

Beside the forms of pigmentation already mentioned the spleen is one of the most common depositories for coal dust (in anthracosis of the lungs). The fine particles of coal are deposited in the spindle cells of the trabeculæ, and the outer coats of the vessels. (*M. F., F. M. Carmine.*) The spleen becomes atrophic as the result of senile change and in cachexia. The organ is reduced in size, loses its elasticity, and is of a brownish color. (*M., M. F. Carmine.*)

On examination with the microscope, sections of the organ show diminution of the pulp, slight increase in the reticulum, and a moderate amount of coal pigment.

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PLATE XXII.

Fig. 1.

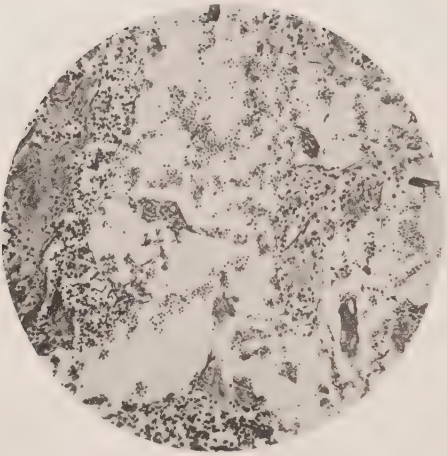


Fig. 2.

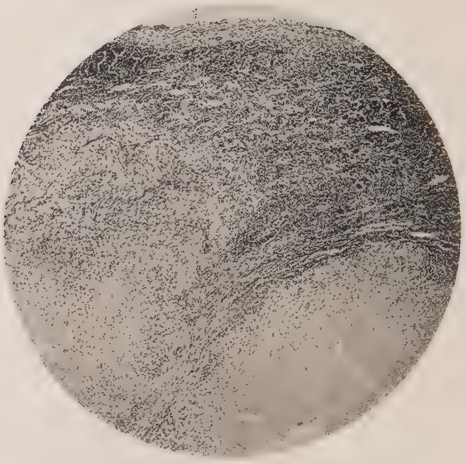


Fig. 3.

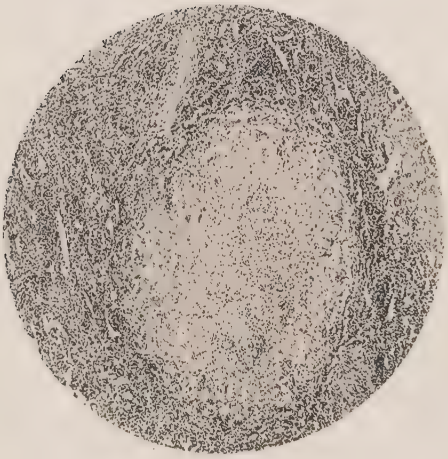


Fig. 4.

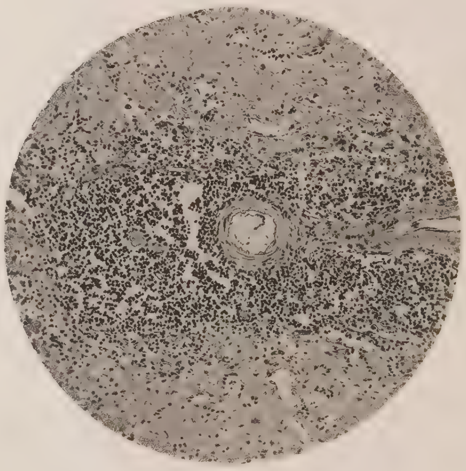


Fig. 5.

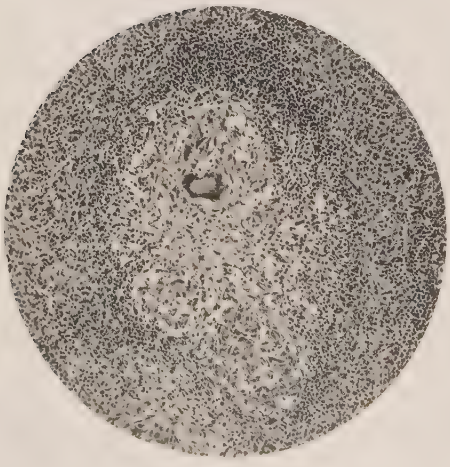
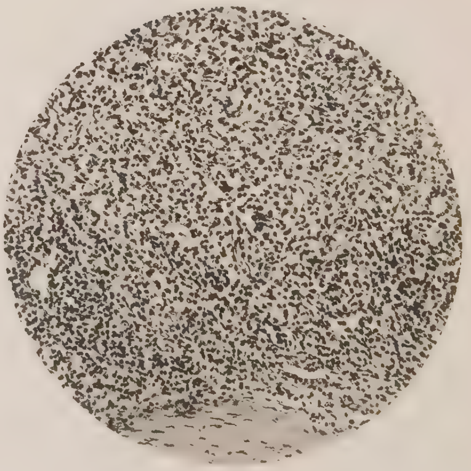


Fig. 6.



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4. Enlargement of the Spleen in Leucæmia.

(Plate XXII., Fig. 6.) In nearly all forms of leucæmia the spleen is enlarged; in the splenic form, excessively. The progress of the enlargement may be divided into a beginning, an acute and a chronic stage. In the beginning the enlarged spleen is comparatively soft, and a considerable amount of fluid may be scraped from the cut surface, which has a dull reddish gray color. The structure of the organ may no longer be distinguished. In rare cases the follicles are particularly enlarged and appear as white lines and dots in the pulp. In both forms hemorrhages into the substance of the organ are common. M. F., F. M., N. S. Sublimate. Embed. Hæmatoxylin. Eosin.

Microscopically the most apparent factor in the enlargement is an enormous increase in the cells of the pulp, as a result of which the trabeculæ are widely separated. (By low power seldom more than one in a field.) The boundaries between the follicles and the pulp are more or less obliterated, the entire section appearing under low power as if composed of one tissue. By high power various cell types may be differentiated. In some rare cases of leucæmia the entire spleen is filled with round cells of uniform size. These are either similar to the cells of the pulp, or resemble the lymphoid elements of the follicles. Generally the round cells in the organ vary in size and are mixed with a large number of polymorphonuclear leucocytes. The hyperplastic pulp occasionally contains giant cells with budding nuclei, and eosinophilic cells, which give to the tissue the appearance of red bone marrow. The capillaries and blood vessels are filled with leucocytes, their walls and the supporting trabeculæ are infiltrated with round cells which obscure the structures. Fresh hemorrhages into the substance of the organ are frequent.

In advanced and late stages of leucæmia the enlarged spleen becomes more dense, its cut surface is smoother, and presents a variegated appearance pro-

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DESCRIPTION OF PLATE XXII.

Fig. 1. Absorption of blood by a lymph node. See p. 192. Low power. Hæmatoxylin. The lymph sinuses are widely dilated and contain groups of spherical, palely stained bodies, which under the *magnifier*, may be recognized, as red blood cells. The darkly stained somewhat larger elements are lymphocytes. To the left a trabeculus.

Fig. 2. Caseous lymphadenitis. See p. 191. Low power. Above the periphery of a lymph node with remains of the lymphoid tissue (*magnifier*). Below an extensive area of diffuse caseation.

Fig. 3. Amyloid spleen (sago spleen). See p. 198. Middle power. Hæmatoxylin. At the center a follicle affected by amyloid transformation. Between the masses of amyloid material fine clefts and openings (capillaries), and cells with spherical nuclei (lymphocytes) (*magnifier*). Surrounding the follicle is unaffected splenic pulp.

Fig. 4. Amyloid spleen (ham spleen). See p. 198. Middle power. Hæmatoxylin. To the right extending toward the center is a trabecula including an artery which at the extreme right is seen in longitudinal, at the center in cross section. The section has included the trabecula just at the point where it changes its direction. The trabecula is surrounded by an accumulation of lymphatic tissue (Malpighian body). The tissue of the splenic pulp above and below is replaced by pale homogeneous masses of amyloid material. The follicle is free from amyloid deposit.

Fig. 5. Tuberculosis of the spleen. See p. 198. High middle power. Hæmatoxylin. Reticulated epitheloidal tubercle. At the center a large giant cell surrounded by large oval and spindle cells with elongated and spherical nuclei. At the periphery of the tubercle a deeply stained zone of lymphocytic infiltration.

Fig. 6. Chronic leucæmia of the spleen. See p. 197. High middle power. Hæmatoxylin. Below a trabecula. Instead of lymphatic tissue, the trabecula is surrounded by a tissue composed of short spindle cells with deeply stained nuclei.

duced by numerous areas and spots of necrosis, and pigmentation which are brilliantly intermingled, especially in the peripheral portions of the organ. The normal structure of the organ is obliterated.

M. F., F. M., N. S. or van Gieson. Zenker. Embed in celloidin. Mallory's connective tissue stain.

Microscopically the place of the pulp is taken by spindle-celled connective tissue, which is especially well developed about the arteries and the trabeculæ, and contains rich deposits of pigment. The necrotic areas are identical in appearance with the infarcted areas in splenic infarct.

5. Tuberculosis of the Spleen.

In general miliary tuberculosis the spleen usually contains large numbers of minute tubercles. It is often difficult to distinguish between tubercles and the Malpighian corpuscles. The diagnosis may usually be made by the great number of the tubercles, the fact that they protrude above the surface, and the detection in some of them of caseation.

M. F., F. M., N. S. Tubercle bacillus stain.

The microscopic appearance shows the usual structure of the tubercles, with occasional caseation. (Plate XXII., Fig. 5.)

6. Amyloid Degeneration of the Spleen.

Next to the liver the spleen is the organ most commonly subject to amyloid change. Usually the follicles are alone affected, when they appear enlarged, transparent and give the typical brown reaction with Lugol's solution (sago spleen). (Plate XXII., Fig. 3.) In a large per cent. of cases, the pulp is primarily affected, the follicles taking on the amyloid material later (ham spleen). (Plate XXII., Fig. 4.) The examination may be made with double knife sections or fresh sections cut on the freezing microtome, or with frozen sections of the organ hardened in M. F. or F. Mallory's connective tissue stain.

Fresh sections examined in salt solution show a network of shiny masses and bands between the meshes of which lie cells, red blood globules, etc. On the addition of acetic acid or potassium hydrate the shiny material remains unchanged. In fresh double knife or frozen sections this material gives the color reactions characteristic of amyloid when treated with iodine or aniline stains (see Liver.) In sago spleen the follicles appear as brown or rose spots in the section.

By high power a few pulp or endothelial cells may be found between the masses of amyloid, indicating that it is deposited about the capillaries and in the finer crevices of the pulp. The trabeculæ are not affected by the change, and appear as clear places in the section. Within them are brown or rose rings, the degenerated vessels. In like manner when the pulp is principally affected the deposit of amyloid is about the vessels and between the pulp cells. The follicles in these cases may be free. Where the process is not too far advanced, the small vessels which contain the first deposits of amyloid material stand out clearly.

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XII. THE SKELETAL SYSTEM.

1. Bone Marrow.—At various ages the bone marrow possesses different characteristics. During the period of development it is very vascular (red bone marrow). The fresh examination (teased preparation in salt solution) of red bone marrow shows it to consist for the greater part of round elements of varying size, most of which contain round nuclei or nuclei with one side pressed in. A few possess horseshoe nuclei. Many of these cells contain coarse granules which stain deeply with eosin (eosinophilic cells). Beside the round cells and eosinophilic cells the bone marrow contains a large number of red blood corpuscles. A certain number of the red blood cells possess nuclei (nucleated red blood corpuscles). These are looked upon as embryonic cells which are later transformed into ordinary red blood corpuscles. This transformation probably occurs by the disappearance of the nucleus within the cell, and not by a process of extrusion, as was previously believed. Here and there a number of nucleated red cells will be seen with nuclei near the periphery, and a certain number of free nuclei may be observed. These last were supposed to indicate the expulsion of the nuclei from the red cells. At present the red cell is supposed to lose its nucleus at the time when it is ready to enter the circulation, the nucleus simply changing into protoplasm like that of the corpuscle. When the period of growth ceases the red color of the young bone marrow is replaced by yellow. The change of color is due to the development within the marrow of fat tissue.

In advancing age the fat disappears and is replaced by a gelatinous material. Under the microscope the marrow consists of a network of capillaries supported by a spindle cell structure, the interstices of which are filled with a clear fluid, and contain a few granulated round cells.

The forms of bone marrow which are associated with different ages may result from pathological changes, as in pernicious anæmia, where the bone marrow of adults returns to the red bone marrow, and in wasting diseases where the marrow prematurely takes on the gelatinous changes of old age. In the second case the substance of the marrow is taken for purposes of nourishment; in the first case the bone marrow is called upon to perform its original function, or its normal function to an excessive extent, and like the bone marrow of infants contains a large number of nucleated red cells. Nucleated red cells appear in the blood of the patient. Large, small, and distorted forms of red globules are present in the marrow (poikilocytosis), and a large number of phagocytic cells containing red blood corpuscles (see Spleen). All diseases of the blood are followed by pigmentation of the bone marrow.

In leucæmia the marrow takes on either a currant juice, or a yellowish green color.

M. F., F. M., or celloidin. Hæmatoxylin eosin.

Microscopically the bone marrow consists of masses of closely packed mononuclear leucocytes, myelocytes, and occasional eosinophiles.

Especially in leucæmia the fresh examination of the marrow shows the presence in large number of pointed hexagonal crystals (Charcot-Neumann crystals). Identical crystals are found in normal marrow, but never in such large number.

2. Pathological Proliferation of Bone.—Disturbance of the growth of bone, especially in the long axis, occurs in the course of certain general diseases.

A bone grows in length by the progressive development of cartilage in the epiphysis, the transformation of this cartilage into bone by the deposition of bone salts and the excavation of medullary spaces, by the growth of capillaries into the newly formed cartilage. Even with the unaided vision the junction of the epiphyseal cartilage with the diaphysis may be divided into zones.

(*a*) At the edge of the cartilage, a fine blue line (proliferating cartilaginous zone).
(*b*) Next to this a very fine white line (the line of calcification).
(*c*) Next to this the red structure of the bone marrow (zone of bone formation).

These three layers are sharply defined from one another and traverse the bone in a straight line at right angles to the long axis.

The rib or femur of a new-born child is best suited for the microscopic examination. Harden in formaline, decalcify, embed in celloidin, or in paraffine and fasten to slide. Stain with alum carmine, counterstain with methylene-blue. The resting cartilage is pale blue, the proliferating blue, the calcified cartilage deep blue, the uncalcified osteoid substance reddish, the calcified bone structure yellow.

The three zones stand out plainly by low power, and may be identified as a blue zone, a narrow line of blue and adjoining bone marrow stained red.

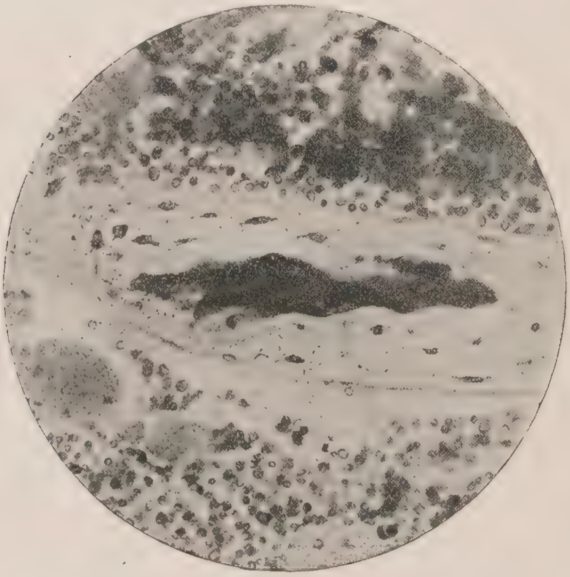
The blue zone is the zone of proliferating cartilage. Beginning with the resting cartilage the margin of the proliferation zone is recognized by an increase in the number of the cartilage cells within the capsules.

As the cartilage cells increase in number they are gradually arranged in parallel rows at right angles to the axis of the bone (so-called zone of parallel arrangement). The capsules about the cells become continuous with the cartilaginous groundwork and the cells increase in size (hypertrophic cell zone). The dark blue line indicates the zone of calcification; in this the ground substance of the cartilage is filled with fine chalk granules. In sections of decalcified bone the chalk is not present, but the area in which it is deposited is indicated by deep blue staining of the hyaline groundwork. The cartilage and cells of this zone do not differ from those of the zone of cell proliferation.

The demonstration of the chalk deposit in the zone of calcification must be made on fresh tissue. For this purpose a thin plate of the epiphyseal junction is cut off with the knife. Under low power the zone of calcification is recognized by its lack of transparency. Under high power the groundwork of the cartilage is filled with fairly large black granules which disappear on the addition of weak hydrochloric acid.

Beneath the zone of calcification is the zone in which the cartilage is invaded by vessels from the bone marrow (zone of bone formation). These vessels produce excavations in the cartilage into which the capsules of the cartilage cells open, liberating the cells which mix with the cells of the bone marrow accompanying the vessels. A row of isolated ribs of cartilage remains, and may be recognized by its deep blue color. (Plate XXIII., Fig. 4.) The bone marrow cells (osteoblasts) arrange themselves in single rows along these ribs of cartilage and transform them into the first bone lamellæ by depositing osteoid substance upon the surface. In so doing they take on a branching form, and are included in the newly formed osteoid substance where they occupy small angular excavations, and form the so-called bone corpuscles. (Fig. 26.)

FIG. 26.



Bone corpuscles in normal bone. Dark calcified bone at center of trabeculus. (Celloidine section. Schmorl's method.)

A new row of osteoblasts forms upon the first layer of osteoid deposit, these deposit a second layer, and so on, the trabeculæ constantly increasing in size (neoplastic bone formation). The newly formed osteoid substance stains red, later it takes on chalk in a form which may not be demonstrated microscopically and then stains yellow. For a considerable period the central cartilaginous portion of the trabecula remains and may be recognized by its deep blue color.

With the formation of new bone, a second process, that of bone destruction, goes hand in hand. The bone grows in length and width by deposits of new bone lamellæ from without, while the medullary spaces are formed from within by excavation of the bone through the agency of giant cells, the osteoclasts. The activity of the osteoclasts may be physiological or pathological. They are usually found in niches and lacunæ which they have absorbed in the osseous structure.

PLATE XXIII.

Fig. 1.

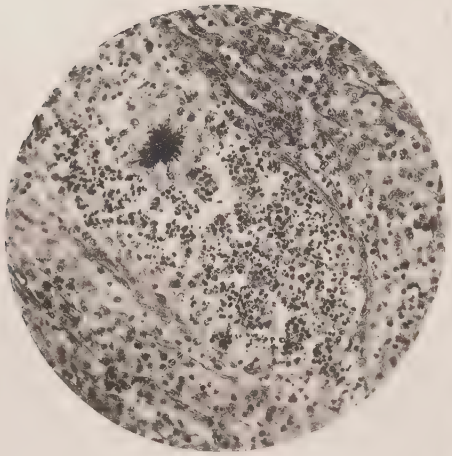


Fig. 2.

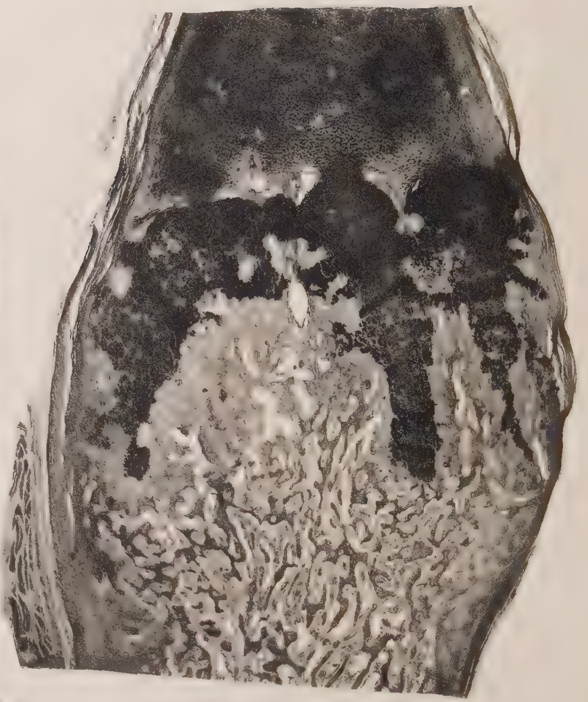


Fig. 3.

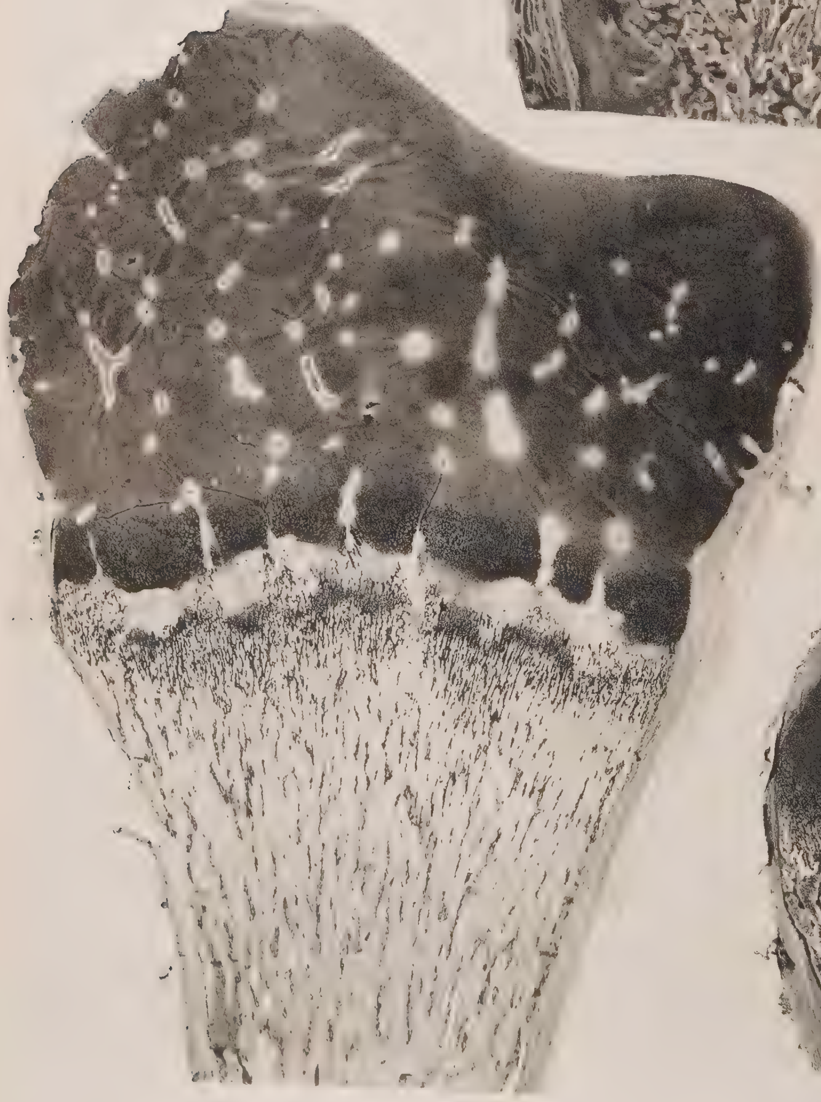
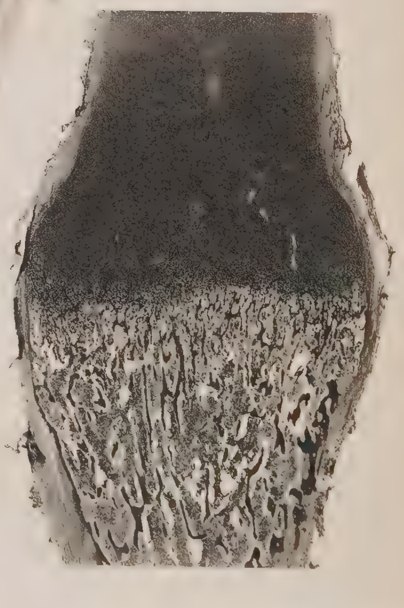


Fig. 4.



Bone sections are best stained with hæmatoxylin, and counterstained with a 2-per-cent. solution of alizarin, or with van Gieson's method (cartilage blue, osteoid substance red, bone yellow). For the demonstration of the bone corpuscles Schmorl's method must be employed (see Technique).

(*a*) Syphilis. Certain disturbances in the growth of the long bones which occur in the syphilitic new-born, may be recognized macroscopically by the great breadth of the white zone of calcification, and the irregular manner in which it is bounded by the cartilage above and the medulla of the bone beneath. Microscopically the proliferation zone is widened and the boundary between it and the calcification zone is very irregular.

The irregularity of the boundary between the calcification zone and the zone of bone formation is even more marked. The capillaries of the medullary spaces, instead of invading the zone of calcification in a comparatively straight line, show marked irregularity. Here and there a capillary breaks through the calcification zone into the cartilage (zone of proliferation).

As a result the formation of the medullary spaces in the cartilage is very irregular. The cartilaginous trabeculæ although formed in sufficient number are very thin, and still more important, the apposition of the osteoblasts is delayed. Thus the junction between the zone of calcification and the point where bone formation occurs is supported only by the thin cartilaginous trabeculæ. (Plate XXIII., Fig. 3.) As a result fractures of the bone at this point are very common.

In some cases the formation of bone is totally disturbed, the bone marrow adjacent to the line of calcification presents a cloudy yellowish appearance and microscopically contains a large number of round cells, in an advanced state of fatty degeneration. Here and there minute foci of necrosis may be found surrounded by round cells.

Separation of the epiphysis in the macerated syphilitic fœtus is a common phenomenon. This usually occurs not at the junction of the calcification and bone formation zones, but on a line with the boundary between the resting and

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DESCRIPTION OF PLATE XXIII.

FIG. 1. Actinomycosis. High power. Hæmatoxylin. Van Gieson. Gentian violet. To the left a colony of the ray fungus with club-shaped bodies, and central deeply-stained radiating filaments. Surrounding the organism closely packed leucocytes (minute abscess), the whole surrounded by a large-celled granulation tissue.

FIG. 2. Rachitic rib, see p. 202. Very low power (Micro-collinear.) Hæmatoxylin alizarin. Above deeply stained resting cartilage. Beneath this large finger-like projections of deeply stained proliferating cartilage. The deeply stained projections of cartilage are surrounded by poorly stained masses (osteoid structure) which are gradually transformed into the more deeply stained trabeculæ below (metaplastic ossification). Through the zone of cartilage proliferation the diameter of the rib is markedly increased (rachitic rosary).

FIG. 3. Syphilis of the femur. Child (osteo-chondritis syphilitica). See p. 201. Hæmatoxylin alizarin. Very low power. (Micro-collinear.) Above deeply stained resting cartilage. At the edge of this the somewhat more intensely stained narrow zone of proliferating cartilage. Beneath this a pale, irregular zone of calcification much wider than normal. Beneath this begins a zone of trabeculæ formation. The trabeculæ are composed of calcified cartilaginous groundwork (deeply stained zone). Beneath this the trabeculæ are enlarged by beginning deposits of osteoid substance upon their surfaces (neoplastic ossification).

FIG. 4. Normal ossification of a rib (cut somewhat obliquely). See p. 200. Very low power. (Micro-collinear.) Hæmatoxylin alizarin. To show the sharp boundary line between cartilage and bone, as compared with Fig. 2, and the few but well-formed trabeculæ which are rapidly covered with osteoid substance as compared with Fig. 3.

proliferating cartilage, which in these cases contains a large number of capillaries. The separation is due to softening of the cartilage induced by the general maceration.

(*b*) Rickets. The presence of rickets is indicated by enlargement and softening of the cartilaginous bony junctions, and the ease with which the softened structures may be bent. In longitudinal sections including the epiphyseal junctions of the long bones or the cartilaginous junctions (rachitic rosary) of the ribs of a rachitic child, a marked widening and distortion of the blue proliferation zone, is apparent. Irregular processes from the cartilage invade the bone marrow. The white line of calcification is absent, or a mere suggestion of it may be made out here and there at the junction of the proliferating cartilage and the bone marrow. The absence of bone salts is best shown by the readiness with which the bone may be cut.

Microscopically the typical arrangement of the different zones is markedly disturbed. The gradual transition from one zone to another is obliterated and the distribution of the zone of cartilaginous proliferation very irregular, in some places very wide, in others narrow.

The bone marrow extends into the zone of proliferation in an unusual manner, in some places extending through the zone of hypertrophic cells into the resting cartilage. (Plate XXIII., Fig. 2.) The masses of cartilage lying between these projections of the medulla show evidence of direct transition into bone (metaplastic ossification). The spaces surrounding the cartilage cells change abruptly into the irregular cavities about the bone corpuscles, the hyaline groundwork takes on an imperfect lamellated appearance and no longer stains with methylene-blue.

The osteoid trabeculæ when formed are unusually thick, present a striated appearance and remain uncalcified for a long period.

Beside this direct transformation into bone, sporadic attempts at regular ossification occur, and here and there trabeculæ may be found with osteoblasts arranged along the edges (neoplastic ossification). The bone marrow contains an unusual amount of spindle cell connective tissue. A considerable amount of soft osteoid structure is formed from the connective tissue of the medulla. The periosteum likewise produces deposits of soft osteoid material.

The fresh examination of rachitic bone is best made by means of a thin plate cut from the enlarged cartilaginous junction of a rib. The proliferating cartilage is easily recognized by its transparent appearance. A considerable amount of glycogen may be demonstrated in the cartilage cells by treating the preparation with Lugol's solution. The newly-formed osteoid substance appears somewhat darker than the cartilage, but is fairly transparent. Some distance away in the bone the first definite indications of calcification may be found in the dark central portions of the trabeculæ.

3. Tuberculosis of Bone.

Tuberculosis most frequently involves the ends of the bones and the joints including the synovial cavities, and coverings of the tendons.

Within the joint cavity the tubercle bacillus produces fibrinous exudation with fibrin formation upon the synovial surfaces. The fibrinous deposits become organized from the surface of the synovia by granulation tissue which contains giant cells, tubercles, and areas of caseation. Fibrin is likewise deposited upon the free surfaces of the cartilages. This is organized from the joint surface by the granulation tissue growing over it from the synovial membrane at the sides. From the surface the connective tissue invades the fibrin and penetrates into the cartilage, causing erosion or even perforation. When the cartilage is once perforated masses of granulation tissue form in the bone marrow beneath it and may be sufficiently extensive to produce separation of the cartilage from the bone.

Tuberculosis involves the bone proper either by extension of a preëxisting tuberculosis to the bone from without, or is primary and takes the form of an infarct of the epiphysis, and involves the joint.

The macroscopic appearances of ordinary tuberculous invasion of bone (bone caries) are variable, caseation, pus formation and granulation tissue predominating. M. F. Decalcify. Celloidin. van Gieson.

Sections through the tuberculous bone show complete absence of the fat which is replaced by tuberculous granulation tissue closely surrounding the trabeculæ. The osteoblasts are displaced, the trabeculæ thin. As the caseation advances the trabeculæ become loosened, detached and disintegrated, leaving small fragments of bone in the necrotic material.

Tuberculosis of the tendon sheaths leads to the production of the so-called rice bodies. On section (*M. F., F. M., N. S. or F. A., embed. Fibrin stain*) these are found to consist of laminated hyaline masses either free from cells or containing a considerable number of spindle-shaped nuclei between the concentric layers. The formation of the rice body can best be understood when the section includes the adjacent tendon capsule. (Fig. 27.)

Fig. 27.

Rice body attached to the sheath of a tendon.

Then the similarity of the rice body to the masses of hyaline fibrin of serous membranes becomes apparent. The hyaline mass is invaded from the sides and base by fibroblasts from the tendon sheath. The cells found in free rice bodies may be migratory cells. A certain number of rice bodies are produced by the hyaline transformation of tuberculous foci in the tendon sheath.

4. Osteomalacia.

Under this name is recognized a disease in which an unusual softening of the bone develops, followed by great deformity of those bones upon which strain is placed either by the action of muscles or the effect of the body weight. The

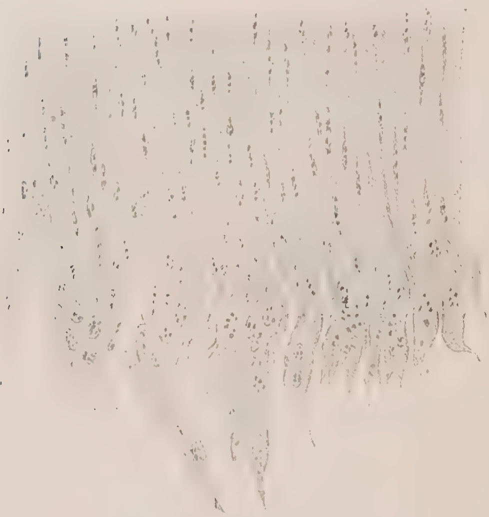
Reference:

F. König. Die spezielle Tuberkulose der Knochen und Gelenke, Berlin (Hirschwald), 1896.

softening of the bone is due to an insufficient amount of lime salts. Whether the insufficiency of salts is due to absorption (decalcification) or to failure of calcification during the regeneration of the bone has not been definitely determined. The more recent investigations indicate that both processes go hand in hand.

Under the microscope the principal evidence of the absence of the lime salts is found in the extreme width of the osteoid margins of the trabeculæ. Fresh material gives the best results and is obtained by cutting fine shavings from the soft bone with a scalpel. In salt solution the osteoid material may be distinguished from the calcified central portions of the trabeculæ by the dark appearance of the latter and the relatively transparent character of the former. When stained with picrocarmine the osteoid tissue takes on a deep red color, the calcified centers a bright yellow. (Plate XXIV., Fig. 1.) A similar color reaction may be obtained with sections hardened for a short time in Müller's fluid, or thin alcohol. The bone corpuscles in the osteoid tissue appear shrunken and atrophic or entirely absent. The lacunary absorption produced by the osteoclasts, as it appears in the formation of normal bone, is absent. If a trabecula be broken out of the bone and stained in alum carmine (containing an excess of alum), a slight reaction takes place and the cavities and lacunæ of the bone corpuscles become injected with the liberated carbonic acid gas and stand out prominently. At the same time a coarse black lattice-like figuration of the osteoid tissue appears (Gitterfiguren Recklinghausen). These are due to widening of the Haversian canals, and cleavage of the interfibrillary crevices. In the calcified portion of the trabecula the lattice-work figuration is absent. Fraying of the edges of the osteoid material may be observed, and occasional areas contain numerous osteoid trabeculæ which possess no laminated structure but appear coarsely striated or mottled. The corpuscles in these trabeculæ are without regular arrangement

FIG. 28.



Degeneration of cartilage, fraying of the surface.

DESCRIPTION OF PLATE XXIV.

FIG. 1. Osteomalacia. See p. 204. Low power. Picro-carmine. The uncalcified osteoid structure stained bright red. Calcified portions yellow. Fat tissue is represented by spherical openings in the red cells of the marrow.

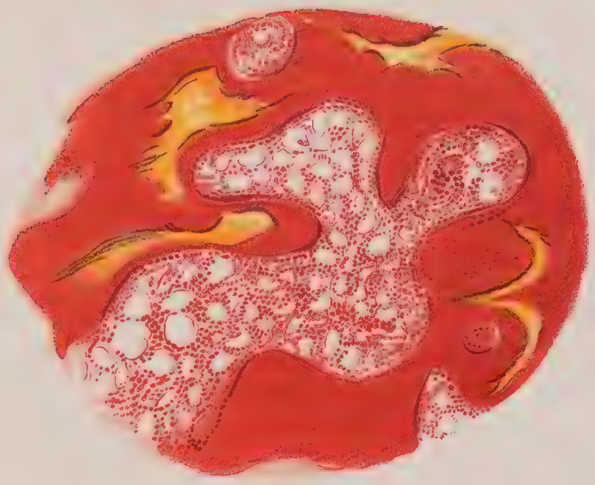
FIG. 2. Pachymeningitis hemorrhagica. Subdural membrane. See p. 209. Stained with carmine. A large number of anastomosing capillaries containing red blood globules. In the openings between these, masses of fine and coarse granular hæmatogenous pigment.

FIG. 3. Rhombic plates and radiating needles of hæmatoidin. See p. 211. High power. Fresh preparation. Hæmatoidin crystals. Sheaves of radiating needles and reddish brown rhombic plates.

FIG. 4. Pigmented nævus. See Tumors. Very low power. Alum carmine. Masses of reddish brown pigment thickly accumulated in the subcuticular layer of the skin. Elevation of the surface and deformation of the papillæ. Cell infiltration in the deeper layers of the tissue.

PLATE XXIV.

1.



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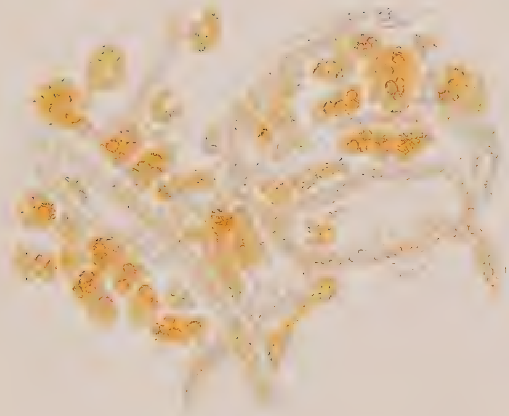

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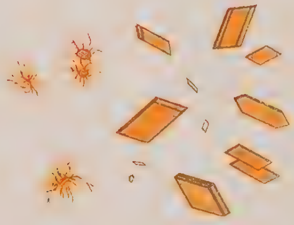

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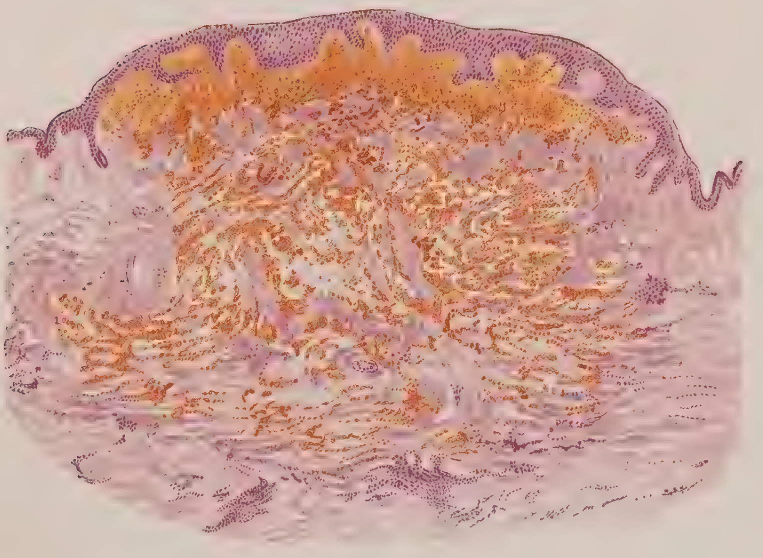


and vary greatly in size and appearance, many being larger than normal. The bone marrow in the proliferating portions has a fibrous appearance, and otherwise contains an excessive number of lymphoid cells.

5. Degeneration of Cartilage.

The joint cartilage in deforming arthritis (arthritis deformans) undergoes varied alterations of structure, which may best be studied in fresh material. For this purpose sufficiently fine sections may be made with the section razor, selecting the yellowish degenerated portions of the cartilage, or by cutting perpendicularly into the cartilage where the surface presents a dull appearance. The examination of the material thus obtained shows a marked fraying of the cartilaginous groundwork at the edge of the section where it includes the dull appearing portion of the joint surface. In the frayed portion the number of the cartilage cells within the capsules is increased (Fig. 28), but this increase, owing to the advanced state of fatty degeneration of the cells, is often difficult to demonstrate. Here and there may be seen deposits of chalk granules in the ground substance. (Test with hydrochloric acid.)

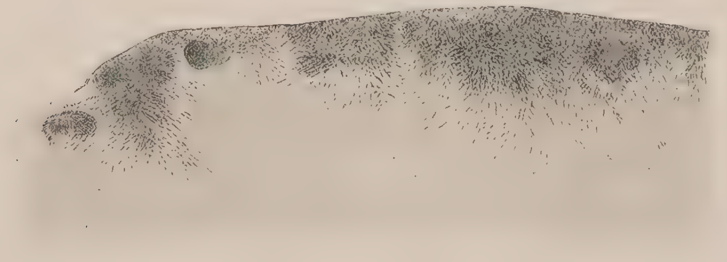
Beside the fraying and partial calcification of the cartilage its structure is invaded by granulation tissue from the joint capsule or the bone marrow. This may be best demonstrated in hardened, decalcified, embedded and stained sections through the cartilage and adjoining structures. The invasion by granulation tissue may lead to total destruction of the cartilage, or it may become transformed into connective tissue, and undergo mucoid degeneration. Transformation of the cartilage into bone occasionally follows the deforming process.

Fraying and cleavage of the cartilage may likewise be observed in the cartilaginous ribs in senile patients. The cartilage appears of a brownish color, and microscopically the groundwork shows splitting and fraying, or breaking down into masses of detritus. Where the degeneration is advanced the cartilage cells are destroyed.

6. Arthritis Urica.

In gout the cartilages of the joints present an intense white appearance and extensive deposits of white, chalky material in the form of nodes project into the joint cavities. Sections of these masses may be made with the scalpel and

FIG. 29.



Deposit of uric acid crystals in the substance of cartilage. (Gout.)

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Schmidt. Neuere Litteratur über die Knochenerkrankungen Lubarsch-Ostertag, Ergebnisse, 1897 u. 1898.

Ribbert. Anat. Unters. über Osteomalacie, Bibl. med. C., Heft 6.

Hanau. (Puerperales Osteophyt), XI. internat. med. Kongress, Rom, 1894.

Bertschinger. Virchow's Archiv, Bd. 147, 1897.

examined under the microscope where the white appearance is found to be due to the presence of dark crystalline deposits which invade the superficial layer of the cartilage. By covering the mirror of the microscope with the hand, these crystalline masses appear by reflected light, when they again take on their white character. By high power the masses are found to consist of accumulations of fine needles (sodium urate) which radiate from the surface into the substance of the cartilage. (Fig. 29.) On the addition of acetic or hydrochloric acid the crystals gradually disappear. Dissolution of the crystals likewise occurs when the tissue is hardened in the usual fixatives. In stained sections where the crystals are dissolved a marked necrosis of the tissue is found in the areas which they have occupied.

Necrosis likewise marks the site of the concretions (Tophi) in the capsule of the joint. A fairly successful *fixation of the crystals may be obtained by hardening the tissue in absolute alcohol.* The sections may be *stained with Vesuvin,* when the crystals are seen to be surrounded by granulation tissue in which they radiate in all directions. If the sections be stained with hæmatoxylin the crystals for the most part disappear, revealing the necrosed tissue which is infiltrated with leucocytes, some of which are in the various stages of disintegration. Numerous foreign body giant cells in the granulation tissue mark the site of the crystals.

7. Repair of Fractures.

All the bone-forming structures participate in the process of repair which follows the dissolution of continuity of a bone. The productive layer of the periosteum, and the bone marrow produce an embryonal granulation tissue, from which the various elements of the new bone are formed. Certain cells become arranged in rows, develop into osteoblasts and deposit osteoid tissue which becomes the nucleus of the bone trabeculæ. These grow in thickness by continued deposition and the inclusion of the osteoblasts in the osteoid material, where they form the cells of the bone corpuscles. By the calcification of these osteoid trabeculæ true bone is formed. The granulation tissue from the periosteum grows in between the ends of the bone and unites with that of the bone marrow and both are converted into bone. Beside this direct formation, an indirect formation of bone occurs from the periosteum. In this case the periosteum produces cartilaginous deposits, or connective tissue, which are later transformed into osteoid and then osseous structure.

The callus, which in the middle stages of the process is excessive, is absorbed by the action of the osteoclasts which reduce it to the necessary dimensions.

M. F., decalcify, F. M. or celloidin. Stain with picrocarmine, methylene-blue and Schmorl's method for the demonstration of bone corpuscles.

XIII. THE SKIN.

Structure.—The skin and mucous membranes consist of three superimposed layers which are known as follows :

An outer layer of epithelium (the epidermis).

A supporting structure of fibrous and elastic tissue which forms the papillæ of the skin, the cutis.

And a substructure of loose connective and fat tissue, the subcutis.

Sections of the outer skin are recognized by : (1) the horny character of the superficial layers of the epithelium, expressed by absence of nuclei in the cells

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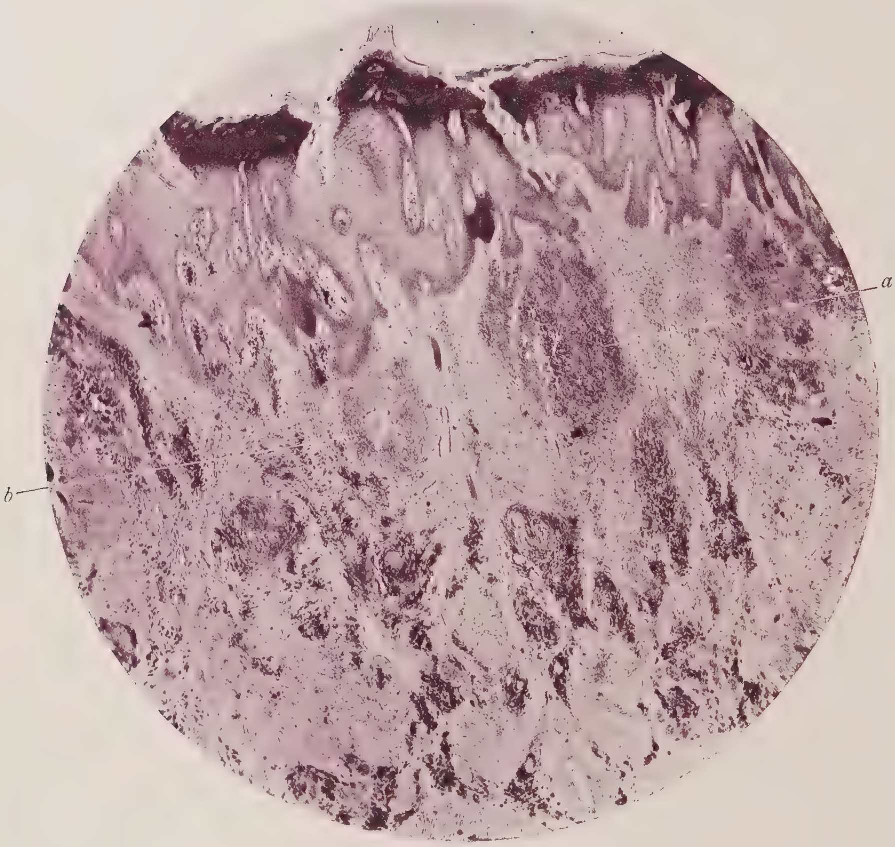
His und Freudweiler. Deutsches Archiv f. klin. Med., Bd. 63, 1899.

PLATE XXIVa.



and granulation (keratin) of the protoplasm; (2) the presence of sweat and sebaceous glands. The former penetrate into the subcutis. (3) Presence of hairs.

1. Repair of Cutaneous Wounds.

Dissolution of continuity of the outer skin is repaired by the formation of a scar, which marks the site of the wound. The scar is at first red, but later changes to white. The process of scar formation is best observed in hardened and embedded sections of the skin and underlying structures at the site of a deep cutaneous (best an abdominal) incision of three or four days' standing. (*M. F., F. M. Hœmatoxylin eosin.*)

Under the microscope the crevices between the sides of the wound are seen to be filled with a coagulum consisting of a fine network of fibrin containing large numbers of leucocytes. The walls of the wound are covered with typical granulation tissue. Where the wound has been contaminated, the granulation tissue and the space between the sides of the wound are filled with pus. In this case bacteria may readily be detected in the secretion from the wound by means of suitably stained cover-glass preparations. If the wound remains uninfected, the leucocytes gradually disappear, the granulations from the sides gradually close in and unite, and the capillaries anastomose. The superficial epithelium grows in a thin layer into the wound opening (Plate XXV., Fig. 3) and ultimately through union of the two sides restores the continuity of the surface. When the red scar is formed we find that the granulation tissue has been replaced by fibrous connective tissue, the line of incision is obliterated and the tissue contains a large number of capillaries which give the skin its red appearance. The white scar differs from the red only by the absence of capillaries and a diminution in the number of nuclei which the tissue contains.

2. Inflammations of the Skin.

The numerous diseases of the skin are associated with characteristic clinical and more or less definite histological appearances. The inflammatory processes are the same in principle as inflammation in other regions. Acute purulent infection (phlegmonic) is associated with accumulations of polynuclear leucocytes which are distributed through the subcutaneous tissue in groups or rows which follow the course of the lymph spaces or subcutaneous veins. Within these accumulations of leucocytes the specific organisms of the infection may be demonstrated. (*M. F., F. M., N. S. or Gram.*)

In chronic inflammations (elephantiasis), accumulations of mast cells, and lymphocytes surround the vessels. The histological alterations of the epidermis in pustule and vesicle formation, and the relation of the glands and hair follicles of the skin to these formations are too varied and special to permit of their being considered in detail.

3. Tuberculosis of the Skin.

M. F., F. M., N. S., F.

A., F. M., Plasma cell stain.

Under low power we note first an unusual appearance of the papillæ which extend deeper than usual into the subcutaneous tissue and branch and fork in

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DESCRIPTION OF PLATE XXIVa.

Lupus of skin. See p. 208. Low power. Unna's plasma cell stain. Beneath papillæ of the skin faintly stained tubercles with giant cells. The deeply stained masses near the bottom of the figure are masses of plasma cells, grouped about the vessels.

an unusual fashion. The cutis contains more cells than normal and here and there the cells are grouped together in the neighborhood of the elongated papillæ.

Under high power the accumulations of cells possess the typical arrangement and structure of epitheloidal tubercles, with giant cells, central caseation, etc. (Plate XXV., Fig. 2.) Attempts to stain the tubercle bacilli in the tubercles are rarely successful. (*Carbol fuchsin.*)

The proliferation of the papillæ is unquestionably produced by the action upon the epithelium of the toxic substances elaborated by the tubercle bacillus. Similar atypical proliferations of the papillæ occur about the margins of chronic ulcers of the skin. In preparations hardened in alcohol and stained after Unna, the presence of so-called plasma cells may be demonstrated. (Pl. XXIVa.) These are located about the peripheries of the tubercles and are distinguished from the connective tissue cells by the deep blue, granulated staining of the cellular protoplasm. (Plate XXI., Fig. 7.)

The plasma cell is considered to be (Unna) a characteristic element of granulomata, and not found in sarcomata.

Beside the plasma cells with their deep blue, coarsely stained bodies, another form of cell of irregular proportions is to be noted whose protoplasm contains bright red granules and a blue nucleus. These elements are mast cells ; normal constituents of connective tissue especially the subcutaneous connective tissue and are increased in chronic irritative conditions of the tissue. (Plate XXI., Fig. 6.) In sections stained with ordinary solutions of methylene-blue the granula of the mast cells take on a deep blue color and where the section fails to include the nucleus of the cell, the blue granules may be mistaken for closely packed cocci, an error which should be avoided.

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v. Marschalkz. Ueber die Plasmazellen. Centralbl. f. allg. Path., X., 1899.

DESCRIPTION OF PLATE XXV.

FIG. 1. Molluscum contagiosum. Low power. Hæmatoxylin. Pouch-like epithelial structure containing coccidæ-like bodies. Central opening communicates with the surface.

FIG. 2. Lupus of the skin. See p. 208. Low power. Hæmatoxylin. Papillæ of the skin above show a typical proliferation. In the cutis and subcutis a considerable number of tubercles. One to the right and below contains two giant cells (*magnifier*).

FIG. 3. Cutaneous wound in process of healing. See p. 207. Very low power. Hæmatoxylin. Cleft in the center is an unhealed laparotomy incision. At the bottom of this cleft a pyramidal accumulation of cells. The epithelium of the surface dips down into the margins of the wound, especially to the right.

FIG. 4. Periphery of an apoplectic cyst, spinal cord. See p. 212. Low power. Mallory's neuroglia stain. At the center a deeply stained structure composed of proliferated neuroglia fibers. To the left apoplectic cyst. To the right the structure of the spinal cord.

FIG. 5. Spinal ganglion cells in anterior pyelomyelitis. See p. 213. Low power. Thionin stain. Ganglion cells with dendrites. Nuclei unstained, nucleoli sharply defined. The Nissl bodies are irregularly distributed through the cells, some dendrites containing a normal number of granules and others, especially the cell above and to the right of the center, show disintegration of the granules in a large portion of the cell protoplasm.

FIG. 6. Periphery of a cerebral tubercle. See p. 213. Middle power. Hæmatoxylin. Above necrosis. At the center and below tuberculous granulation tissue containing giant cells.

PLATE XXV.

Fig. 1.

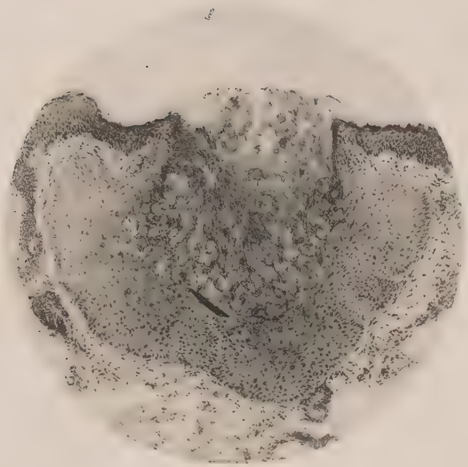


Fig. 2.

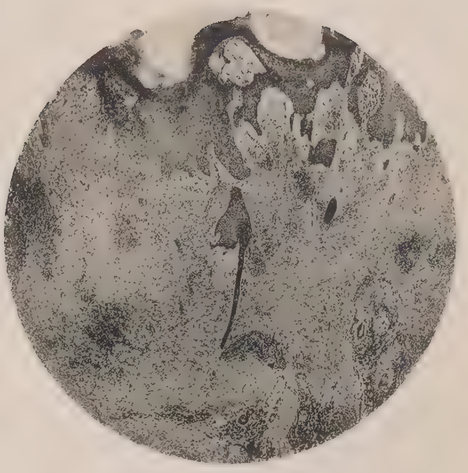


Fig. 3.

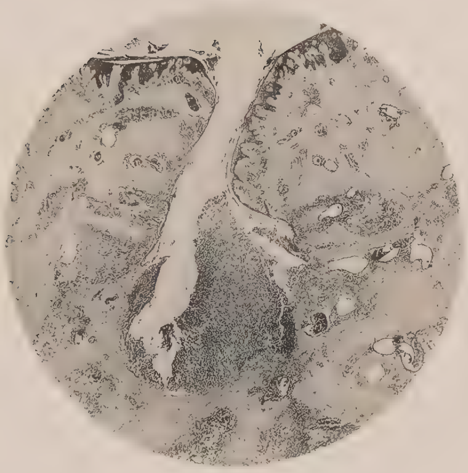


Fig. 4.

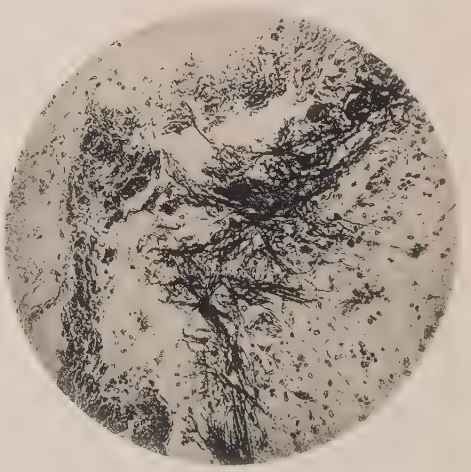


Fig. 5.

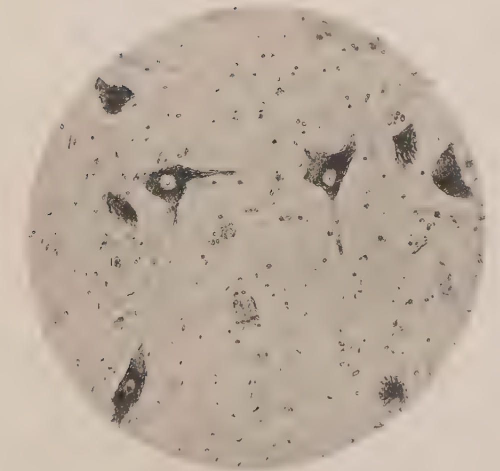
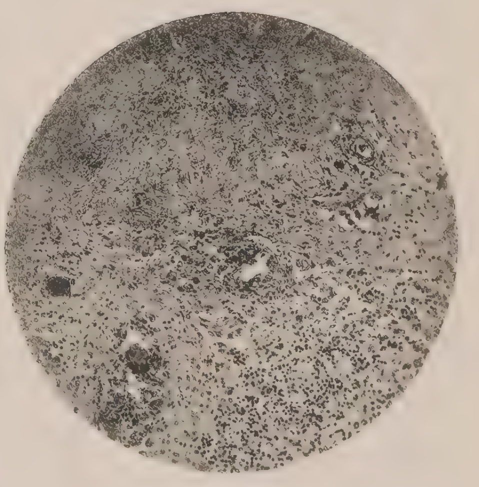


Fig. 6.



XIV. THE NERVOUS SYSTEM.

1. Pachymeningitis Hæmorrhagica.

The inner surface of the dura mater in certain diseases (following trauma, alcoholism, dementia paralytica) shows spots of brownish yellow discoloration. The discolored surface may often be stripped up with the forceps, when the stripping should be spread out on a slide and examined in salt solution. Under the microscope the thin membrane consists of a fine network of capillaries surrounded by spindle cells. These last named elements are filled with brownish yellow hæmatogenous pigment which is likewise deposited in the endothelial cells of the capillaries and lies free in the openings of the capillary network. (Plate XXIV., Fig. 2.)

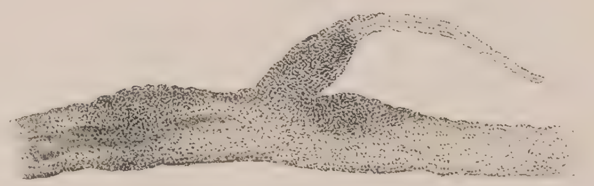
The presence of this pigmentation marks the site of previous hemorrhagic deposits on the inner surface of the dura which have been organized by a highly vascular connective tissue. Under the action of the organizing connective tissue the coloring matter of the extravasated blood becomes transformed into pigment and is taken up by the cells. The richness of the capillaries in these areas occasions frequent hemorrhages on the old site which occasionally elevate the pigmented membrane and produce small hæmatomata.

2. Leptomeningitis.

The ordinary acute and chronic forms of leptomeningitis present no unusual characteristics.

In the purulent form the pia covering the convolutions and fissures of the convexity presents a yellowish discoloration. Under the microscope the discoloration is found to be due to an excessive accumulation of polynuclear leucocytes. The presence of organisms in this pus may be easily demonstrated by means of the usual stained cover-slip preparation. In sections of the membranes (*M. F., celloidin, Weigert's fibrin method*), the character and distribution of the micro-organisms may be determined. These are diplococci identical in appearance with diplococci of fibrinous pneumonia, and are commonly located in the leucocytes (meningococcus intracellularis).

Fig. 30.



Isolated vessel of the pia with tubercles (fresh preparation).

The microscopic examination of the tissue is of greater importance in tuberculosis of the meninges. Even when the diagnosis has been determined by the presence of tubercles about the sylvian fissure, the distribution and character of the tubercles can only be ascertained with the aid of the microscope. For this purpose a small, square piece of the basal pia should be cut out with the shears and removed with the adherent vessels from the brain substance. The piece of membrane is rinsed energetically in salt solution and a few of the small vessels may be detached, spread out on a slide and examined in salt solution, or they may be stained with carmine and examined in glycerine.

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Under the microscope the vessels show occasional thickenings, usually unilateral, and by high power in a favorable preparation the thickening is seen to be localized without the muscular coat of the vessel. Most of the tubercular nodules are therefore located in the adventitia (Fig. 30) although extensive inflammation of the intima is occasionally noted with infiltration of leucocytes in the coats of the vessel. The crevices between the membranes contain rich deposits of fibrin and accumulations of leucocytes.

M. F., F. M., hæmatoxylin and tubercle bacillus stain.

F., or F. A., celloidin, fibrin stain.

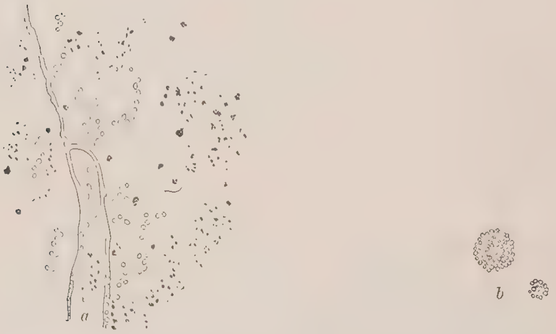
3. Softening of the Cerebral Substance.

Embolic occlusion of a cerebral vessel leads to anæmia and death of the tissue within the area which it supplies, exactly as vascular occlusion occasions destruction of tissue in other organs (kidney, spleen).

Great caution must be exercised in the diagnosis of thrombosis in the brain as post-mortem clotting is especially common in the cerebral vessels.

Macroscopically the affected tissue is recognized by the great softening it has undergone (white softening). A similar softening of the nervous tissue, especially of the cord, results from post-mortem disintegration, so that a microscopic examination of the softened material is necessary before the intravital character of the change can be recognized. Where the change has occurred intravitam, the softened mass contains a large number of characteristic elements, the so-called compound granule cells, or fat globule cells. To determine the presence of these cells a small amount of the softened cerebral tissue is pressed out under a cover-glass until the layer is sufficiently thin to permit of observation. By low power the tissue appears nearly transparent and is marked by fine dark lines and minute black dots. By high power the dark dots are found to be somewhat larger than ordinary cells and to consist of masses of fat droplets closely packed together about a central clear space in which, after treatment with acetic acid, a nucleus may be demonstrated. The nucleus is round, fairly large, and contains but a small amount of chromatin. These cells are either fixed tissue cells in an advanced state of fatty degeneration, or migratory cells which have taken up the fat droplets. (Fig. 31.) The dark lines seen by low power are resolved under high power into small vessels which owe their distinct delimitation in the tissue to long spindle elements arranged along the outer coats of the vessels. These are the endothelial cells of the peri-vascular lymph channels, which likewise contain large numbers of fat droplets, giving them under low power a dark appearance. The fat droplets are for the most part derived from the disintegrated myelin sheaths of the nerves. In frozen sections a characteristic reaction may be obtained by treating the section for a few moments (5 minutes) with a 2-per-cent. solution of osmic acid, after which all

Fig. 31.



a, fresh mashed preparation from softened focus in the brain, dark spots compound granule cells; *b*, compound granule cells by higher power.

the fat droplets become intensely black. If the softening is advanced, fragments of the degenerated myelin sheaths and axis cylinders, the latter swollen and distorted, may be found.

Where the softening is due to trauma, or hemorrhage (arterio-sclerosis, aneurism) into the substance of the brain or cord, the softened material is at first a distinct red, later a more yellowish color, produced by the extravasated and then disintegrated blood. In old areas of softening due to hemorrhage the remaining coloring matter of the blood is found (under the microscope) in the form of the usual brownish yellow pigment, deposited in cells which lie in the neighboring perivascular lymph spaces. In the necrotic material, rhombic plates (Plate XXIV., Fig. 3) and needles of hæmatoidin mark the site of the hemorrhage. In hardened sections through fresh localized hemorrhages the vessels of the surrounding tissue adjacent to the periphery of the hemorrhage, are found to be surrounded by cylindrical mantles of blood. This is due to absorption of the extravasated blood by the perivascular lymph channels, which open into the hemorrhagic area. Simple and hemorrhagic softenings of the brain substance occur in the neighborhood of brain tumors and abscesses, and are due to the pressure exerted on the nervous substance by the foreign mass. Toxic irritation occurs in the case of abscesses, which interferes with the nutrition of the cells and produces degeneration. Traces of widespread degeneration are likewise found in the brains of general paralytics. Mashed preparations taken from the junction of the white and gray matter of the brain, show the endothelial cells of the lymph spaces filled with fat droplets. The fat droplets in these cases are often of deep yellow color and could be mistaken for pigment granules, except for their spherical form and high refractive index. Deposits of blood pigment in the cells of the adventitia and endothelium of the perivascular lymph spaces do occur in cases of advanced general paralysis, for which reason the character of the two deposits must be carefully determined. Hyaline thickening of the smaller cerebral vessels is likewise associated with this disease (hardened sections) which with the traces of brain softening constitute the coarser microscopic changes. The earlier stages of the disease, in which the ganglion cells are altered, and the gradual disintegration of certain nerve tracts require special methods of investigation.

4. Encephalitis Neonatorum (Virchow).

The brain substance of the newborn and infants in the first few weeks of life contains, when the tissue is examined in fresh mashed preparations, a large number of fat droplet cells. These are not the result of pathological change, as the tissues of other organs in infants and the newborn contain an excessive amount of fat in the form of droplets deposited in the cells. In other cases the substance of the brain contains numerous deep yellow spots, occasionally softened and surrounded by hemorrhage (encephalitis). In hardened sections, *M. F.*, *F. M.*, *N. S.*) marked accumulations of round cells about the vessels, and necrosis of the tissue indicate the distribution of the softened brain substance.

These localized softenings occur most commonly in the brains of syphilitic infants.

5. Proliferation of Neuroglia.

The brain and cord contain three classes of tissue.

1. The specific ganglion cells and their prolongations (neurones).
2. A special supporting tissue for the nervous system, the neuroglia, which consists of cells possessing large numbers of fine prolongations (spider cells) which form a fine network between the nerve structures.

3. The connective tissue of the vessels which is much less developed than the neuroglia and consists of spindle cell tissue which surrounds the vessels and includes the perivascular lymph spaces.

When the nerve substance is destroyed, whether by disturbances of circulation, inflammatory or pure degenerative processes, the neuroglia of the adjoining tissue shows evidence of marked proliferation. As restitution of the neurones but seldom occurs, this proliferation represents an attempt on the part of the neuroglia to repair the defects of the nerve substance. By this means, the defects which remain after absorption of the softened cerebral substance, are partially repaired or where small may be filled in by the growth of neuroglia.

Formaline. Further treatment of hardened fresh material for the demonstration of neuroglia according to Mallory or Weigert. For the demonstration of the myelin sheaths according to Bolton, or after Marchi, or ordinary staining with van Gieson's stain.

If a section of a small area of softening [be hardened and stained according to Mallory, the neuroglia of the surrounding tissue which appears as fine filaments, is found at the periphery of the cyst to swell into a mass of thick fibers. (Plate XXV., Fig. 4.) It is, of course, possible, that the connective tissue, especially of the vessels of the pia, aid in the restoration of traumatic defects. Occasionally the ependyma of the ventricles, in chronic hydrocephalus (in children as early as one or two years), show a fine granulation of the surface not unlike the appearance of minute tubercles. In sections hardened and stained according to Mallory these minute protrusions are found to consist of tiny proliferations of neuroglia (glioses) which have broken through the covering of the ependyma and project above the surface.

The epithelium of the ependymeal surface is displaced, but occasionally proliferates between the projections of neuroglia, forming minute cysts and gland-like structures.

6. Tuberculosis of the Brain.

The minute bodies of granular ependymitis may readily be mistaken for true tubercle formation which occurs in the ependyma and on the surface of the ventricles especially of the posterior horns.

Formaline. F. M., N. S. or tubercle bacilli stain.

The small elevations of the surface and accumulations of cells beneath the surface fail to resemble the typical tubercle by the absence of caseation and giant cells, but the specific factor, the presence in the nodes of tubercle bacilli, may be demonstrated. The cells of these tuberculous nodes are probably not derived from the neuroglia, as they fail to react to the specific staining methods of Mallory and Weigert.

Having determined that the tuberculous infections of the brain are usually located in the pia and ependyma, it is only necessary to state that chronic tuberculosis of the brain almost invariably has its beginning in a tuberculosis of the pia.

The so-called solitary tubercles (tuberculoma) of the brain are usually located in the depths of the fissures, where they have their starting point in the folds of the pia from which they invade the brain substance.

The relation of the tuberculous mass to the pia may often be demonstrated in sections. The process begins by the formation of closely packed miliary

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tubercles, which break down, and form one large area of caseation, at the periphery of which the fresh tubercles may be seen (conglomerate tubercle). (Plate XXV., Fig. 6.)

7. Degeneration of the Ganglion Cells.

In softening of the brain substance the ganglion cells undergo marked fatty degeneration, a condition which must be distinguished from senile pigmentation in which the cells take on spherical granules of pigment. These pigment granules are turned black with osmic acid but are to be distinguished from fat by their arrangement in crescent form against one side of the cell, and their regularity in size, neither of which characteristic appears in fatty metamorphosis of the cells. A rare form of degeneration is calcification of the ganglion cells, in which the protoplasm of the cell is filled with minute granules of chalk, giving the cell a stiffened appearance. An understanding of the finer alteration of the protoplasm of the ganglion cells we owe to recent observations (Nissl).

Formaline. F. M. Stain according to method on page 159.

By means of special staining methods a better insight into the structure of the protoplasm may be gained. The ganglion cells of the cerebrum, and of the anterior horns of the cord afford the best examples. In preparations prepared after Nissl, or properly stained with thionin, the protoplasm of the ganglion cells is seen to be filled with closely packed granules which have taken on a deep blue color. The size, number and distribution of the granules vary in different regions, but as a rule the ganglion cells of the same region contain a similar number and present a similar distribution of the granules (well seen in the large motor ganglion cells of the anterior horns of the cord).

Experimentation has demonstrated that certain toxic substances (tetanus toxine alcohol) or separation from its peripheral prolongations, produces changes in the cell which principally affect the granulæ. These show various forms of disturbance, in some cases being irregularly clumped together in the cell, or widely separated, or partly or totally destroyed. (Plate XXV., Fig. 5.) At the same time the ganglion cell becomes swollen with distortion and displacement of the nucleus. After a certain period of time the cell may regain its normal appearance and function, or where the action of the toxine or trauma has been too severe, the nucleus is destroyed and with it the cell perishes.

8. Tract Degenerations of the Spinal Cord.

The white substance of the spinal cord is composed of nerve fibers, grouped in independent systems. These systems of fibers are grouped together according to their origin and the direction in which they run, and are of two kinds, motor and sensory. (See diagram in explanation of Plate XXVIII.) The principal tracts and their origins are as follows:

1. The anterior pyramidal tracts, composed of motor fibers from the ganglion cells of the anterior horns of the same or opposite side.
2. The lateral pyramidal tracts, composed of motor fibers from the opposite hemisphere of the cerebrum to the motor ganglion cells of the anterior horns of the same side.
3. The posterior tracts, sensory, made up of fibers from the posterior spinal ganglia which enter the cord through the posterior nerve roots and form the columns of Goll and Burdach, which extend upward, terminating in the terminal nuclei. From the ganglion cells of the terminal nuclei a second series of tracts arise and extend to the cortex of the cerebrum. A second group of sensory fibers enter the cord through the

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posterior nerve roots. These do not extend upward in the posterior columns, but join the columns of Clarke, on the same level at which they enter.

4. From these last-named fibers are derived the lateral cerebellar tracts, composed of sensory fibers which extend upward to the cerebellum, crossing in the crura cerebelli. From here a series of systems of nerve fibers connect with the cortex of the cerebrum.

Destruction of the areas from which these nerve fibers spring, for instance, the motor areas of the left side, is followed by degeneration of the tracts downward, through the basal ganglia, the medulla and the entire length of the cord, so that the course of the tract may be accurately determined.

Fig. 32.

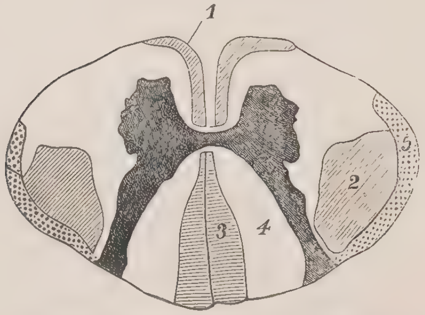


Diagram showing the tracts of the spinal cord: 1, anterior pyramidal tracts; 2, lateral pyramidal tracts; 3, columns of Goll; 4, columns of Burdach; 5, lateral cerebellar tracts.

A similar degeneration occurs when the tract is forcibly interrupted in its course, in which case that portion of the tract degenerates which is separated from its origin, the motor tracts from the brain peripherally, the sensory from the periphery toward the center.

Formaline hardening. The degenerated tracts may be demonstrated by hardening the cord after Marchi when the degenerated portions appear black, due to the effect of the osmic acid on the free myelin detritus which they contain. In like manner the degenerated tracts may be demonstrated by differential staining methods where the portions appear lighter (Weigert Pal's method) than the healthy tracts. As an increase of the neuroglia occurs in the degenerated tracts these may be demonstrated by employing special methods which stain the neuroglia (van Gieson, nigrosine, Mallory) in which case the degenerated appear darker than the healthy tracts.

(For hardening and staining, see page 57.)

The principal forms of tract degeneration of the cord are:

(*a*) **Tabes Dorsalis.** (**Posterior Sclerosis.**)—Cross sections of the cord in advanced tabes reveal a degeneration of the fibers of the root zones in the lumbar region higher up in the columns of Burdach and in the columns of Goll in the cervical region. (Plate XXVI., Fig. 5.) The sensory nerve fibers entering the cord in the lumbar region turn upward in the outer regions of the posterior tracts but are gradually pushed toward the median line by the fibers which enter the cord above, so that they ultimately (in the cervical region) come to form the columns of Goll. By tracing the degeneration through the cord we may readily see that it affects a certain group of sensory fibers. On examination of the posterior nerve roots degenerated fibers are found, showing that the process begins in the spinal ganglia.

The greater the number of posterior roots involved, the greater will be the extent of the degeneration area in the posterior columns until the entire tracts of Goll and Burdach are involved. Space will not permit a consideration of the complications of tabes and the alterations which these produce in the cord.

(*b*) **Ascending and Descending Degenerations of the Cord.**

In the affections known to clinicians as spastic spinal paralysis or paraplegia, and amyotrophic lateral sclerosis, the motor tracts are involved, *i. e.*, the anterior pyramidal and lateral pyramidal tracts.

REFERENCE:

Schmaus. Lubarsch-Ostertag, Ergebnisse, Abt. III., 1894.

PLATE XXVI.

Fig 1.

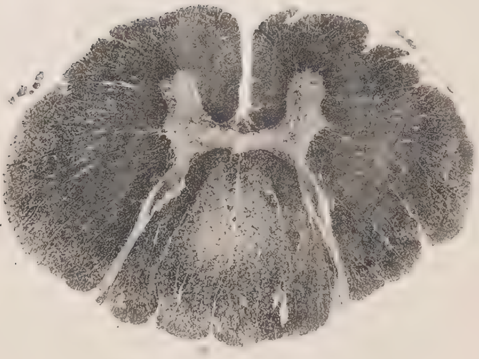


Fig. 2.

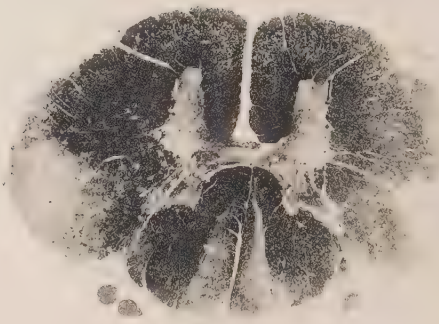


Fig. 3.

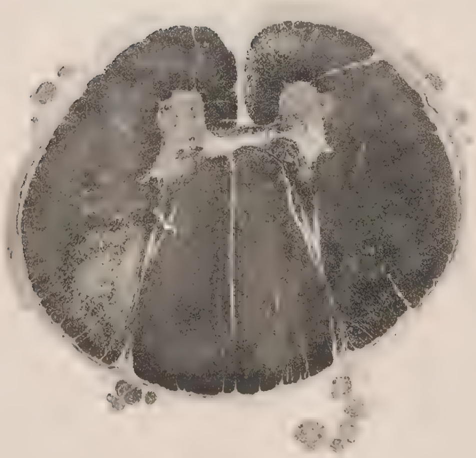


Fig. 4.

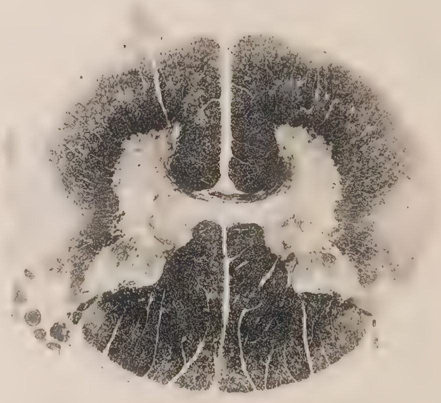


Fig. 5.

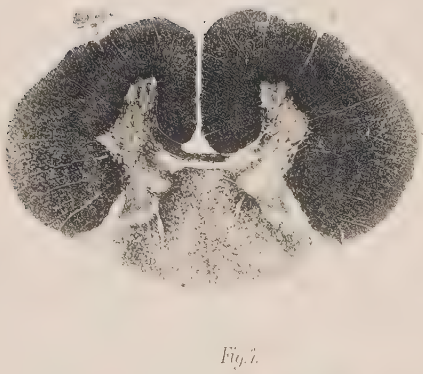


Fig. 6.

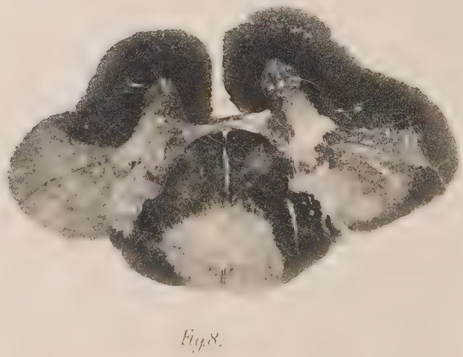


Fig. 7.

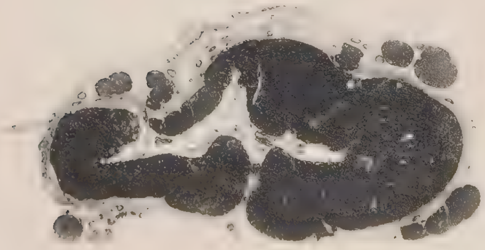
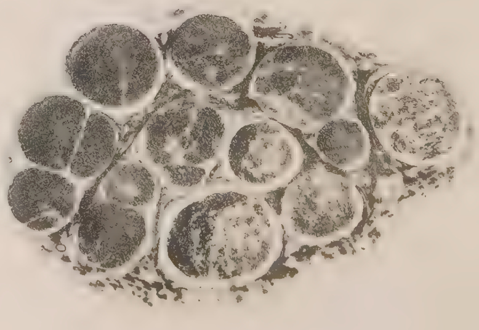


Fig. 8.



An even more definite degeneration of these tracts results when unilateral pressure is brought to bear upon the cord, as occasionally occurs in hemorrhage into the inner capsule of the cord, in which case the fibers of the anterior pyramidal tract of the same and the lateral pyramidal tract of the opposite side degenerate. (Plate XXVI., Fig. 3.) Complete dissolution of continuity in the cord is followed by degeneration of all the motor tracts beginning at the site of the trauma and extending downward. A similar degeneration of the sensory tracts, *i. e.*, the posterior and lateral cerebellar tracts, extends upward from the point where the cord is severed. (Plate XXVI., Fig. 2.) In sections of the cord stained with nigrosine, a certain number of unusual spherical bodies are formed in the degenerated areas especially in tabes. In fresh mashed preparations, these bodies may be readily detected and when treated with iodine are found to give a blue reaction for which reason they are known as corpora amylacea. These are supposed to be formed from the degenerated myelin substance of the nerve sheaths.

(*c*) **Amyotrophic Lateral Sclerosis** is a disease affecting the ganglion cells of the anterior horns of the cord, and associated with degeneration of the pyramidal tracts (Plate XXVI., Fig. 4), and pseudo-hypertrophic degeneration of the muscles. The character of the degeneration of the muscles is identical with that found in progressive muscular distrophy, and consists of hypertrophy of some fibers, atrophy of others, increase in the connective and fat tissue, loss of longitudinal and transverse striation, and increase in the number of nuclei of the muscle fibers.

9. Disseminated Multiple or Insular Sclerosis.

In this disease a variable number of irregular gray bodies appear in the white matter of the cord and brain. (Plate XXVI., Fig. 6.) On microscopic examination these are found to be localized areas of degeneration, which differ from the tract degenerations by the irregular manner in which they include the

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DESCRIPTION OF PLATE XXVI.

FIG. 1. Beginning ascending degeneration following crushing of the cord. See p. 215. Very low power. (Micro-collinear.) 60 mm. Weigert-Pal's method. Lightly stained area of degeneration in the anterior portion of the posterior columns.

FIG. 2. Ascending degeneration (advanced stage). See p. 215. Very low power. (Micro-collinear.) Weigert-Pal. Light areas of degeneration corresponding with the columns of Goll and the lateral cerebellar tracts.

FIG. 3. Unilateral descending degeneration. Lesion in the right side of the cortex above the decussation of the pyramidal tracts. See p. 215. Very low power. (Micro-collinear.) Weigert-Pal. Light degeneration areas corresponding with the anterior pyramidal tract on the right side and the lateral pyramidal tract on the left side.

FIG. 4. Amyotrophic lateral sclerosis. See p. 215. Very low power. (Micro-collinear.) Weigert-Pal. Pale areas of degeneration in both lateral pyramidal tracts.

FIG. 5. Posterior sclerosis (tabes). See p. 214. Very low power. (Micro-collinear.) Weigert-Pal. The area of the degeneration is confined to the posterior columns (Goll and Burdach).

FIG. 6. Multiple sclerosis. See p. 215. (Micro-collinear.) Weigert s method. Irregular pale areas of degeneration not limited to any special tracts.

FIG. 7. Syringomyelia. See p. 216. Very low power. (Micro-collinear.) Weigert's method. Irregular cavity more extensive on the right side than the left. At the center the central canal (*magnifier*). (This figure should be reversed, the anterior portion being below.)

FIG. 8. Degeneration of a peripheral nerve (section of musculo-spiral just before the muscular branch is given off. Lead palsy). The pale fibers in the bundles to the right and left are those derived from the muscular branches, which are atrophic. (Figs. 1–7 same magnification.)

tissue, without regard to the functional distribution of the nerve fibers. The axis cylinders in the degenerated foci remain intact, even after the myelin sheaths have disappeared. By special staining methods a marked increase of the neuroglia may be demonstrated. The walls of the vessels show marked hyaline degeneration. The pathogenesis of this disease is obscure.

10. Syringomyelia.

Beside a simple dilatation of the central canal of the cord (hydromyelia) a special disease exists in which an extensive hollowing out of the center of the cord occurs, probably by softening, and the space thus formed usually communicates with the central canal and is filled with fluid. (Plate XXVI., Fig. 7.)

The long axis of the excavation is in the length of the cord and the cavity usually lies in the region of the posterior columns, and is surrounded by proliferating neuroglia. The cavity may be incompletely lined with a layer of epithelium which is continuous with the epithelium of the central canal. The excavation of the cord is probably produced by an inflammatory proliferation of neuroglia with secondary softening of the newly formed tissue, a predisposition to the condition being incomplete closure of the primary fissure of the cord produced by certain disturbances during fœtal development.

Many points in the pathogenesis of syringomyelia are still unsettled, especially the derivation of the epithelium in the cavity, which according to some observers is developed from epithelial cells, which find their way into the excavation from the central canal.

XV. THE FEMALE GENITAL ORGANS.

1. Erosion of the Cervix.

The surface of the cervix is formed into papillary projections upon which rests a layer of cylindrical epithelium. The nuclei of the cells of the epithelial covering lie near the base, the protoplasm is filled with a clear material (mucus). The mucous membrane contains tubular or alveolar glands which are lined with epithelium of the same type as that covering the surface. These normally extend into the superficial muscular layers of the cervix, and commonly undergo cystic dilatation (ovula Nabothi).

Erosions of the cervix appear as crescentric or irregular, bright red spots surrounding the opening into the cervical canal.

M. F., F. M., N. S.

The section should include one side of the cervical canal.

The microscopic appearance of the eroded tissue varies exceedingly. The alteration which first attracts attention is the unusual development of the mucous glands, which extend over the vaginal portion of the cervix, whereas in the normal uterus they cease at the external orifice.

The squamous epithelium of the vagina in this area is replaced by cylindrical. The transformation of the vaginal mucous membrane into cervical mucous membrane may be traced at the margin (simple erosion). Occasionally the surface is covered with villous projections (papillary erosion), or cystic enlargement of the glands (ovula Nabothi) leads to the formation of visible vesicles in the mucous membrane (cystic erosion). Lastly, the entire mucous membrane may be missing, in which case the denuded connective tissue structure develops into granulation tissue, and is transformed into a true ulcer (granulating erosion).

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2. Endometritis.

The mucous membrane of the uterine cavity presents three principal characteristics.

1. It rests directly upon the uterine muscle without the inter-position of a submucosa.

2. The supporting structure of the mucosa is well developed and consists in youth of a structure of round cells which after puberty are transformed into short spindle elements.

3. The entire extent of mucous membrane contains closely placed perpendicular tubular glands, which, as well as the mucous surface, possess a definite membrana propria and are lined with a simple layer of ciliated cylindrical epithelium. The nuclei are not arranged along the base of the cells, but are placed at various elevations in the cell protoplasm. Many of the glands branch at their lower ends and extend laterally into the muscular structure, and because of the closely packed cell nuclei appear in sections more deeply stained than the superficial segments of the glands. The glands take a tortuous or more commonly wavy course.

Chronic endometritis, a condition in which the mucous membrane is markedly thickened, is associated with various diseases of the uterus, but more particularly occurs where the uterus is misplaced. A common accompaniment of the inflammation of the mucous membrane is increased pain and bleeding during menstruation or hemorrhage at irregular intervals between the menstrual periods. As hemorrhage is a common symptom in malignant disease of the uterus, it is often necessary to diagnose this condition by means of scrapings from the uterine cavity. *The material obtained is best hardened in formaline or M. F. If the pieces are sufficiently large they may be cut on the freezing microtome, but where small all the fragments should be imbedded together in celloidin, or paraffine and transformed into celloidin films later.* N. S.

The microscopic examination shows the following alterations of the tissue.

1. An excessive increase in the connective tissue groundwork, by which the glands are widely separated and collapsed. The cells of the connective tissue remain of normal character but increase somewhat in length. Blood pigment and leucocytes are scattered through the proliferating tissue (endometritis interstitialis). If infection occurs, a purulent infiltration follows, often of sufficient intensity to entirely obscure the interstitial structure. The epithelium of the glands is desquamated and is seen lying with leucocytes in the gland lumenæ (endometritis purulenta).

2. In the majority of cases the glandular structures of the mucous membrane show an equal, when not greater, increase than the interstitial tissue. First the glands are increased in length in proportion to the thickening of the mucous membrane, beside being twisted in a corkscrew or tortuous fashion. (Plate XXVIII., Fig. 1.) Secondly the gland lumenæ show great variation in diameter, being in some places nearly closed by pressure from the proliferating tissue without and in others widely dilated (endometritis glandularis). Occasionally complete closure of the gland lumen occurs, followed by the formation of cysts (endometritis cystica). (Plate XXVIII., Fig. 1.) The principal point to be observed in all these changes is that the epithelium, with the exception of flattening from pressure in the cystic dilatations, undergoes no alteration and remains a simple layer of cylindrical epithelium, although the position of the nuclei in the cells may lead to appearances suggestive of multiple layer formation.

This is due to the nuclei in different cells lying at different elevations. A similar appearance which might be mistaken for true multiple layer formation occasionally appears in thick sections where, owing to the length of the nuclei, they are sectioned in different planes, producing the effect mentioned. The proliferation of the glands may be so excessive as to nearly obliterate the intervening connective tissue, without indicating any malignant tendency of the growth.

Only when the entire section is composed of closely packed and richly branching gland structures, without sufficient connective tissue to form a limiting membrana propria, should the probability of malignancy be considered.

The condition just described is commonly the forerunner of the so-called malignant adenoma in which (without visible alteration of character in the epithelium) the glandular structures invade the muscular layer of the uterus.

If this invasion of the muscular wall, especially if the glands extend to the region of any of the larger vessels, the diagnosis of malignancy should be made, although at the same time it must be borne in mind that certain glands normally extend into the superficial muscular layer so that the presence of isolated sections of glands in the muscle does not indicate the occurrence of malignant growth.

The appearance of the invaded tissue in adeno-carcinoma is sufficiently different, although the process begins in the glands. First of all the malignant gland-like structures possess no membrana propria, and the appearance of the epithelium at once distinguishes it from that of the normal glands. The carcinomatous epithelium varies greatly in size, the protoplasm stains deeply, and the nuclei of the cells vary within the greatest possible limits of form, size, amount of chromatin and number. Multiple layer formation is a constant occurrence and the lumena of the pseudo-glands are commonly obliterated by the proliferating epithelium. Ultimately the masses of epithelium fail to show any resemblance to the original gland structure. The process of cell division in the normal gland occurs with the line of segmentation perpendicular to the gland wall, whereas in the carcinomatous pseudo-glands the line of cleavage may lie in any plane.

The mistake of confusing the above characteristic appearances of adeno-carcinoma with the alteration of the uterine mucous membrane following menstruation or abortion, must be avoided.

In these last-mentioned conditions the gland epithelium appears unusually enlarged, and the nuclei contain more chromatin than usual. Here and there the cell boundaries are obliterated, producing syncytial-like masses of protoplasm. All of these changes are part of the process of regeneration of the destroyed superficial and glandular epithelium. The formation of multiple layers in the glands is rare and the preservation of the typical structure of the mucous membrane should afford sufficient protection from a false diagnosis of malignancy.

3. Endometritis Tuberculosa.

Tuberculosis of the uterine mucous membrane may occur as a slowly disseminating tuberculosis, or may affect the entire uterine cavity with a rapid caseating process. Combinations of the two processes are not wanting. The evidence of the disseminated form is not uncommonly found in scrapings from the uterus (*M. F.*, *F. M.*, *N. S.*), where the mucous membrane contains numerous epitheloidal tubercles, which are distributed without regard to the structure of the mucosa. The superficial epithelium is usually intact. Caseation is rare. The bacilli are not readily demonstrable.

In caseous endometritis the sections (M. F., F. M., tubercle bacilli stain) show a diffuse caseation, extending from the surface of the uterine cavity into

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Amann. Kurzgefasstes Lehrbuch der mikr. gynäk. Diagnostik, Wiesbaden, 1897.
Cullen. Carcinoma of the Uterus, D. Appleton, 1901.

the mucous membrane. Here and there the caseation dips down into the healthy portions of the membrane through infection of a gland. The demonstration of the bacilli in the caseated material and the gland openings is readily accomplished by proper staining methods.

4. The Changes in the Uterine Mucous Membrane Accompanying Menstruation and Pregnancy. The Placenta.

The diagnosis of an existing or preëxisting pregnancy by the examination of cast-off tissue or scrapings from the uterus is complicated by the similarity of the changes in the uterine mucous membrane during menstruation and at the beginning of pregnancy.

During ordinary menstruation the mucous membrane becomes thickened, the glands are more convoluted than usual and the vessels are engorged. The epithelium of the surface and occasionally in the glands is detached from its base by more or less extensive hemorrhage beneath the cell layer.

The blood clots found in the uteri of patients who have died during active menstruation, commonly contain fragments of connective tissue which include segments of blood vessels. This rather indicates that the superficial connective tissue is disintegrated during menstruation, but the observation is open to the criticism that the separation of the tissue may have been post-mortem.

In a certain number of patients suffering from endometritis entire casts of the uterine cavity are passed at the menstrual period (dysmenorrhœa membranacea).

These casts are in the form of three-cornered sacs of tissue having an opening at each corner. The nature of the cast varies in different cases, sometimes consisting of fibrin, or a mixture of fibrin and mucus and occasionally of layers of altered uterine epithelium. The walls of the sac are perforated with minute holes which correspond to the openings of the uterine glands.

Occasionally the cast is composed of the superficial layer of the uterine mucous membrane which has been desquamated en masse. In these cases the connective tissue of the desquamated membrane shows marked alteration, the cells being larger, oval and more closely packed than normal. No noticeable changes occur in the epithelium of the glands or in the blood vessels. A considerable number of leucocytes are present, lying between the enlarged connective tissue cells. The alterations of the mucous membrane in this case are exactly like those which occur in the beginning of pregnancy, the characteristic feature being the transformation of the connective tissue cells into large oval elements, the so-called decidual cells. In early abortion (first month) the extruded sac presents no microscopic difference from the casts thrown off in membranous dysmenorrhœa. The exterior is rough, the cavity smooth. If the sac is intact and is opened with great care, it is usually possible to locate the tiny embryo as a small lump beneath the surface near the base of the triangular cavity. The lining of the sac is unaltered where it extends over the elevation, except at the apex where it is slightly thickened. If sections be made through the elevation (*harden in sublimate, embed in celloidin*) perpendicular to the surface, the embryo, its coverings, and their relations to the lining of the uterine cavity may be studied. (*Stain hœmatoxylin eosin.*) Ordinarily the sac is torn during the abortion and the embryo is lost. In such cases it is necessary to make the diagnosis of pregnancy by investigation of the sac

Reference:

Gebhardt. Subepitheliale Hämatome. Zeit. f. Geburtsch. u. Gynäkol., Bd. 32, 1895.

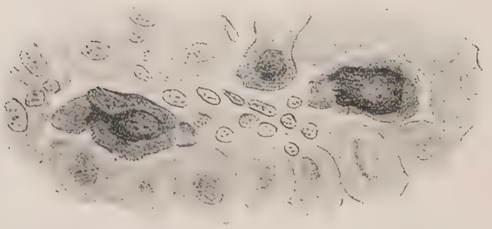
which consists of the desquamated superficial layer of the thickened uterine mucous membrane (decidua vera).

M. F., F. M., N. S., Perpendicular sections through the wall of the sac.

In the very early stages of pregnancy the microscopic appearance of the aborted decidua vera is identical with the tissue composing the sac in dysmenorrhœa membranacea. In both cases the connective tissue cells show the characteristic transformation into closely packed oval cells, the glands are enlarged, tortuous and lined with cubical epithelium, and a certain number of leucocytes invade the tissue. The superficial epithelium is flattened. In such cases without the clinical history it is impossible to make a differential diagnosis by the microscopic examination of the decidua vera.

Somewhat later in the course of pregnancy (second month) further changes occur in the decidua, which render the diagnosis possible. The connective tissue cells, as before, are transformed into decidual elements but these are now larger, present a pale, vesicular appearance and are closely packed together. (Plate XXVIII., Fig. 3.) The protoplasm is clear and pale, the nuclei round or oval and contain very little chromatin. The cells are sharply differentiated from the dilated vessels, which consist of simple tubes of endothelium.

FIG. 33.



Decidua serotina, deeply stained cells of the chorion surrounded by pale cells of the decidua.

The changes in the mucous membrane are not the same in the various regions about the embryo. For this reason, if one has the opportunity of investigating a young embryo (second or third month) in situ, it will be seen at once that the formation of the characteristic decidual cells is practically confined to the superficial layer of the uterine lining. In this layer the glands gradually disappear, forming what is known as the decidua compacta. In the deeper layers of the membrane, the connective tissue cells are enlarged, but do not show the vesicular dilatation of the true decidua cells. The glands in the deeper layer are preserved and show marked alteration, being very plentiful and tortuous with dilated lumenæ, into which project papillary elevations covered with wide cylindrical or cubical epithelium. The epithelium of the surface, where the decidua reflexa has not united with it, is markedly flattened.

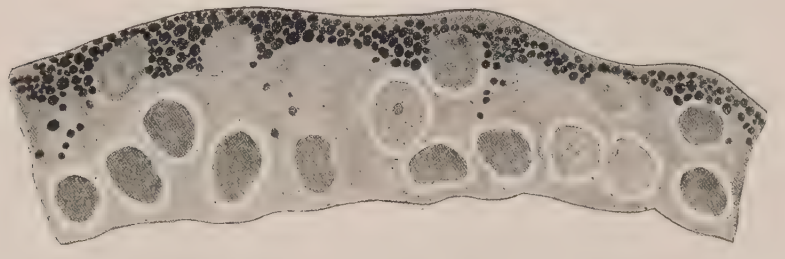
The epithelial cells of the glands when examined fresh, are seen to contain numerous fat droplets beside spherical hyaline bodies (drops of glycogen) which turn brown when treated with iodine. The glycogen in the cells may likewise be demonstrated in tissue hardened in alcohol. (See page 52.) The description thus far given refers to the decidua vera. If the embryo is lost and fragments of the decidua vera remain, the diagnosis of pregnancy may be made by the microscopic examination of fragments removed with the curette. The characteristic changes in the glands, although they appear somewhat later in the decidua vera than in the serotina, and disappear in the later stages of pregnancy when the tissue becomes stretched, still serve to facilitate the diagnosis.

Where the transformation into decidual cells is incomplete, or where, as regularly occurs in advanced pregnancy, they have lost their characteristic appearance, or when the embryo has been extruded and the processes of involution have been installed, the examination of scrapings, where these consist of decidua vera only, does not always enable us to make a positive diagnosis of pregnancy. Under such circumstances, the probability of pregnancy may be asserted. In so doing, it must be remembered that similar changes of the uterine mucous membrane occur in extrauterine pregnancy, and that this condition must be included among the possibilities in the case.

Positive evidence of uterine pregnancy is only obtained when the scrapings include portions of the decidua from the region where the ovum has become fixed (decidua serotina). As in the decidua vera, the principal change in the uterine covering at this point consists of the transformation of the connective tissue elements into decidual cells. But here we find for the first time, between the decidual cells, a new form of cell which makes its appearance after the fixation of the ovum. These new-comers are spindle-shaped or oval, of regular form and about the same size as the decidual elements, but are at once distinguished from them by the dark coloring of their protoplasm and their deeply-stained, irregularly shaped nuclei (Fig. 33). The great variation in the forms of these cells renders a short description of their charactristics difficult. The nuclei are sometimes large, sometimes small, either round oreoval, and commonly of unusual irregular form.

Occasionally cells smaller than the decidual cells are seen and large elements with multiple nuclei much greater than the decidual cells are frequent.

FIG. 34.



Normal syncytial covering of villus from very young embryo (osmic acid) showing arrangement of nuclei and distribution of fat drops. (ASCHOFF.)

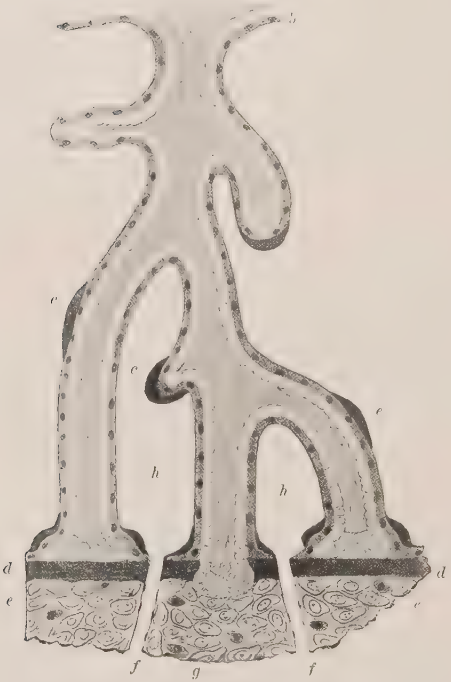
All of these deeply stained elements are fœtal cells derived from the epithelial coverings of the chorionic villi.

The recognition of these cells is of the greatest importance as they may be found in the decidua serotina months after the extrusion of the embryo and when the processes of resolution have destroyed the characteristic appearance of the decidual cells. (See Plate XXVII., Fig. 1.)

Where the fœtal coverings of the embryo are present in the tissue under examination the diagnosis of existing or preëxisting pregnancy is simple and certain. These are recognized by the projecting chorionic villi which consist of central axes of fœtal connective tissue containing vessels and covered with layers of epithelium. These villi may retain their vitality for long periods after the death of the embryo, or detached villi may be found in the blood clots. (Plate XXVIII., Fig. 2.) The villi should be searched for in casts by picking out the white particles with a needle and freeing them from the blood by rinsing in salt solution. Where a villus is thus obtained, its appearance under low power is that of a branching structure. To determine that the mass is not fibrin, acetic acid should be added, when the structure of the villus becomes apparent and is seen to possess a central connective-tissue trunk and branches covered with epithelium in which plentiful fat droplets appear. (In the syncytium the fat is regularly arranged; see Fig. 34.)

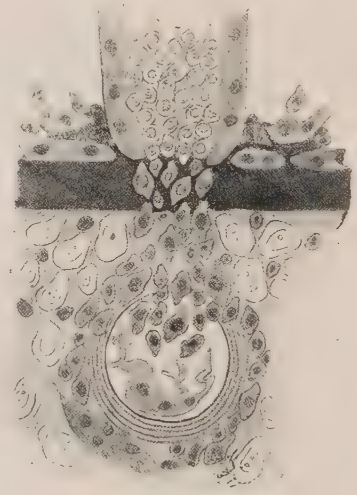
To obtain a clear idea of the structure of the placenta and its relation to the uterine wall, the student must have at his disposal sections of a young embryo, with the uterine mucous membrane surrounding it (*sublimate, celloidin, H. E.*). According to the latest investigations, the ovum, after it enters the uterine cavity, bores into the superficial layers of a transverse fold of the mucous membrane and here develops. From the thin superficial layer of the mucous membrane which covers the ovum, is formed the decidua reflexa. As the embryo grows, the decidua reflexa, from which surface of the ovum, after a certain period, the villi disappear, becomes continuous with the layers of the embryonic sac. The accompanying development of the membrane beneath the site of fixation of the ovum, the decidua serotina, shows marked deviation from the above. The chorion soon develops projections (villi), which consist of a central connective tissue structure, covered with a double layer of epithelium, the inner of which is a single row of pale cubical cells (layer of Langhans) and the outer forms a confluent covering of darker protoplasm in which numerous deeply-stained nuclei are embedded (syncytium). (Fig. 35.) Both of these layers are derived from the fœtal ectoblast (Minot, Aschoff). Wherever the villi come in contact with the decidua serotina, a marked proliferation of the fœtal epithelium occurs, especially the cells of the layer of Langhans, which break through the syncytium and invade the decidua or spread out on its surface (Fig. 36). These migrating chorionic cells penetrate into the serotina, the superficial layer of which contains no gland structures, and break through the walls of the widely dilated superficial vessels, by which means a communication is established and the blood flows into the intervillous spaces between the chorion and the surface of the serotina. Later in the course of pregnancy (third or fourth month) the villi are more closely arranged, the entire placenta forming a pad which is perforated by numerous openings, through which the maternal blood enters or leaves. At this stage the surface of the serotina is covered by a layer of fœtal ectoderm and the placental space no longer comes in direct contact with the decidual surface. After separation two layers may be distinguished on the maternal surface of the placenta. The superficial (next the uterus) is composed of maternal decidua, and between this and the intervillous spaces a second layer composed of fœtal tissue derived from the coverings of the villi. Between these two layers and on the intervillous surface of the fœtal layer, extensive layers of hyaline material, in part derived from the cells but for the most part altered fibrin deposits. (Fig. 35.) In the mature placenta

FIG. 35.



Structure of placenta (diagrammatic). *a*, layer of Langhans; *b*, syncytium; *c*, fibrin deposits on villi; *d*, fibrin layer of Nittabuch; *e*, decidua; *f*, maternal veins opening into *h*, intervillous blood spaces; *g*, fœtal cells in the decidua.

FIG. 36.



Invasion of blood vessel by cells of the chorion (diagrammatic).

PLATE XXVII.

Fig. 1.

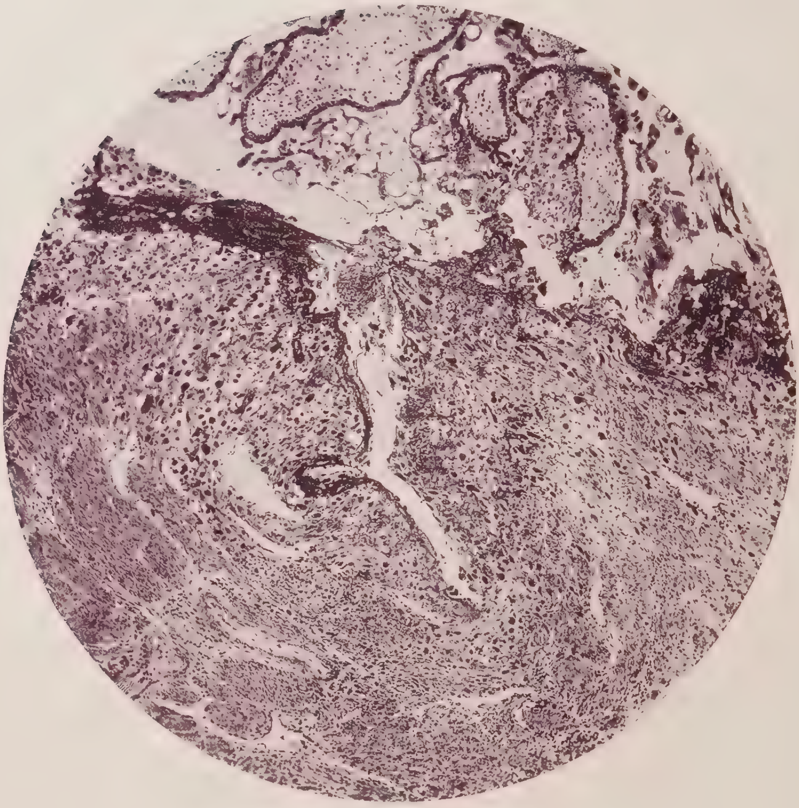
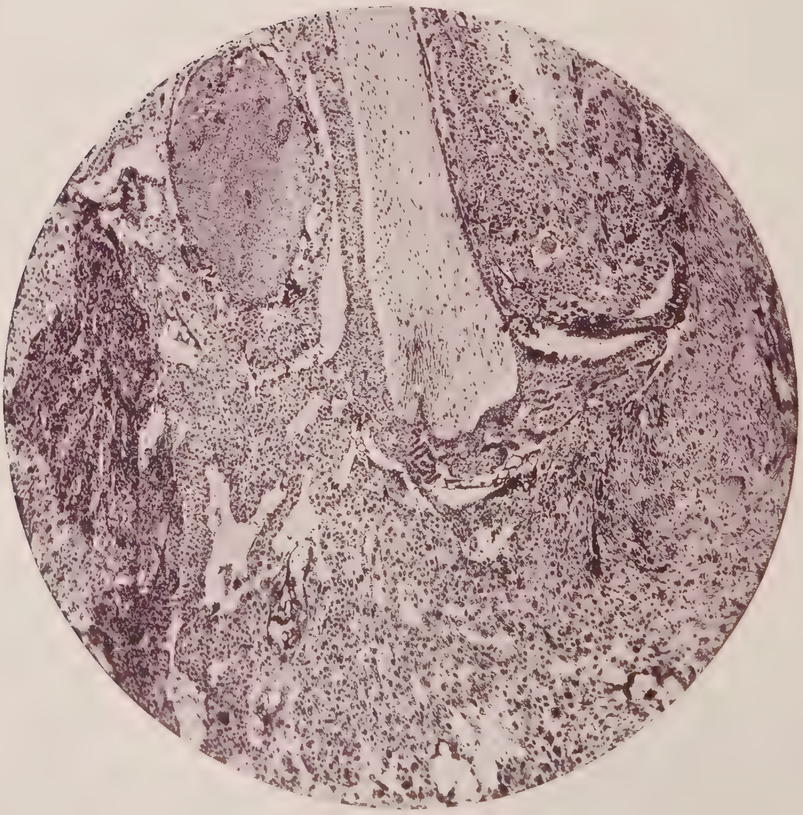


Fig. 2.



the villi are covered by a single layer of epithelium, the syncytium, the layer of Langhans having disappeared.

If, after the interruption of pregnancy, the ovum is not immediately extruded, but remains in the uterine cavity, the chorionic villi become necrotic, but may still be recognized in the blood clot by their characteristic structure. In hardened sections a certain number of the cells of the epithelial covering will be found which have retained their staining qualities, the remainder being in a condition of advanced necrosis.

Among the most frequent pathological changes in the placenta are the so-called placental infarcts. These appear as white patches in the placenta beneath the chorion and include the entire thickness of the organ, with the serotina, and are often extensive. The previous belief that these infarcts indicated syphilitic infection of the fœtus, has not been sustained. The true cause of the condition has not been definitely ascertained.

M. F., F. M., or celloidin. N. S. or van Gieson.

Zenker. Celloidin. Mallory's connective tissue stain.

Under the microscope the intervillous spaces in the infarcted area are seen to be filled with material which unites the closely packed villi. In the central portions of the infarct, the placental tissue shows marked evidence of necrosis, often affecting complete villi. The character of the intermediate substance varies in different infarcts. In some it consists of proliferated chorionic epithelium embedded in hyaline material, in others it consists of pure fibrin which bridges over the intervillous spaces with bands and coarse network. Red and white blood corpuscles fairly well preserved may be found near the periphery of the infarct, but in the central portions these fall victims to the general necrosis, their previous existence in this locality being indicated by deposits of pigment. The condition or conditions which have produced the stasis of the blood current in the infarcted area may no longer be determined. Syphilis of the placenta leads to great thickening of the villi especially the ends, the result of excessive hyperplasia of the connective tissue stroma, through which the volume of the placenta is increased.

The syphilitic changes in the vessels of the umbilical cord are more characteristic and of greater diagnostic importance. The adventitia of the vessels

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DESCRIPTION OF PLATE XXVII.[1]

Fig. 1. Section of uterine wall in hydatidiform mole. See p. 224. Low power. Hæmatoxylin, van Gieson. Above sections of the hydropsical villi, without central vessels. The two epithelial layers of the chorion may be well distinguished. Extending from the smaller villus at the center above an atypical proliferation of syncytium. To the left upon the surface of the uterine wall a deeply stained mass of fibrin. Extending into the tissues from the surface great numbers of large and deeply stained chorionic cells. Below sections of the uterine vessels.

Fig. 2. Chorion carcinoma. Extensive mass of proliferating epithelium containing chorionic villus. See p. 259. Low power. Osmic acid preparation stained with safranin. Extending from above the connective tissue stroma of a villus, the epithelial coverings of which on the right side are intact. On the left side a thick layer of proliferated epithelium. Extending from this and from the end a massive proliferation of epithelium which fills the entire field. Above and to the left an oval mass of fibrin. In the proliferated epithelium irregularly shaped cavities. Just below the cleft in the end of the villus a mass of syncytium rolled together. Here and there in the epithelium are chorionic giant cells.

[1] Fig. 1 is a photograph of a section of the original hydatidiform mole described by Marchand. Zeitsch. f. Geburtsch. u. Gynäk., Bd. 32, 1895.

Fig. 2 is from a case of chorion carcinoma described by Professor Marchand in the same journal, Bd. 39, 1898. The author is indebted to Professor Marchand for both these specimens.

and commonly the media and intima are infiltrated with round cells. This condition, due to syphilis, must not be confused with an obliterating process which affects many of the vessels in the placenta after the death of the fœtus, and which possesses the typical characteristics of an endarteritis obliterans.

In general, disseminated, miliary tuberculosis, or in uterine tuberculosis of the mother, the placenta may become infected, the tubercles being located in the connective tissue stroma of the villi.

Hydatidiform mole is a condition of the placenta which almost invariably leads to abortion. The investigations of Marchand have shown that the principal factor in this condition is not the dropsical, occasionally mucoid enlargement of the villi by an excessive proliferation of the fœtal ectoderm which covers them.

M. F., celloidin, van Gieson or alcohol, celloidin hæmatoxylin, glycogen stain, or Zenker celloidin. Mallory connective tissue stain, or Hermann, celloidin. Mallory's iron hæmatoxylin or safranin.

On microscopic examination of the mole, the enormous proliferation of the epithelium of the villi becomes apparent. (Plate XXVII., Fig. 1.) This occurs not alone at the ends of the villi, but over their entire surface may be seen large, irregularly shaped cells and masses of protoplasm containing nuclei. A rich amount of glycogen is found in the cells of the layer of Langhans and the syncytium contains numerous fat droplets. The invasion of the serotina by placental cells, is, in these cases, excessive. (Plate XXVII., Fig. 1.) Occasional cells of similar appearance occur in the stroma of the villi and are supposed to represent a pathological invasion of the mesoblastic stroma by the chorionic epithelium (Neumann).

The microscopic examination of the mucous membrane of the uterus, directly after childbirth shows that the deep layers even at the placental site, have been preserved. The fundi of the uterine glands are found in this layer and from these remnants of gland structure the formation of a new layer of epithelium for the surface, and the regeneration of the glands, occurs. The placental site (decidua serotina) and the rest of the uterine cavity (decidua vera) show marked differences in structure and composition. The placental site is distinguished by the number of large placental giant cells, which infiltrate the decidua and even the superficial muscular coats. These cells are entirely absent in the decidua vera. They are derived from the covering epithelium of the villi and invade the serotina about the middle of pregnancy, and disappear during the first eight days of the puerperium. They are commonly arranged about the uterine vessels.

Occasionally the membranes of the fœtus are intimately attached to the lining of the uterine cavity (decidua vera), and must be removed with force.

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In such cases Aschoff has observed an abnormal proliferation of the chorionic epithelium in the region of the decidua vera. The decidua in this region is markedly atrophic, containing no glands, and in some spots is entirely missing, the fœtal coverings resting directly upon the exposed uterine muscle.

5. Pyosalpinx.

The Fallopian tube is composed of: (1) a mucosa, the inner surface of which is thrown into deep folds and covered with a simple layer of ciliated cylindrical epithelium. (2) A submucosa of connective tissue, and (3) a muscularis of two layers, the inner circular, the outer longitudinal.

A definite muscularis mucosa is not present but a suggestion of such a structure is occasionally seen in a few longitudinal muscle fibers under the mucosa. In cross sections the papillary projections of the mucosa and the depressions between them give the tube an appearance as if lined with glands.

The principal inflammatory affections of the Fallopian tube are chronic purulent salpingitis and tuberculosis. Both of these conditions commonly lead to closure of the tubes with a resulting dilatation and accumulation of pus or caseated material (pyosalpinx).

M. F., F. M., N. S.

In both cases the wall of the tube when examined in section with the microscope is found to be thickly infiltrated with round cells, which penetrate into the muscular coats and lie in masses about the vessels. In tuberculosis of the tube the thickened mucous membrane may contain numerous tubercles or the entire lining of the tube may be involved by a diffuse caseating process.

6. Tubal Pregnancy (Ectopic Gestation).

The early diagnosis of extrauterine pregnancy is of great importance as the condition almost invariably leads to severe hemorrhage from the tube and the formation of a retro-uterine hæmatocele, and is not uncommonly followed by sudden exodus. The conditions which lead to tubal impregnation are not definitely known, chronic catarrh of the tube, and abnormal development of the ovum in some cases being considered the determining factors.

Kinking of the tube in its uterine portion, and adhesion to the surrounding parts are more commonly the result of the impregnation than the cause of it.

The general view that the mucous membrane of the tube reacts in tubal impregnation in the same way as the uterine membrane, is held by one of us (Aschoff) to be false.

In contradistinction to the conditions in the uterine mucous membrane, the structure of the tube early in the pregnancy shows no or very slight transformation of the connective tissue into decidua, even at the site of fixation of the ovum, although later in the pregnancy a considerable development of decidua occurs in the other portions of the tube. This lack of decidual tissue in what should be the decidua serotina, continues during the course of embryonic development, and does not afford the ovum the cushion of thickened mucous membrane which it finds in the uterus. The results of this anomalous condition are very serious. First, no decidua reflexa is formed, the ovum, in its growth, simply lifting up the superficial layers of muscle and mucous membrane. Second, the mucous membrane at the site of fixation of the ovum, is rapidly invaded by the chorionic cells of the embryo, which penetrate into the muscular layers, open up the vessels and so weaken the wall of the tube that as the ovum develops it thins, stretches, and ultimately ruptures.

Rupture of the tube produced in this way occurs almost invariably during the second, and at the latest in the third month of pregnancy. The peritoneum may be ruptured with the tube, in which case the embryo is extruded into the peritoneal cavity. Rupture of the muscular coat alone is followed by hemorrhage from the eroded vessels of the placental site. The thinning and rupture of the tubal wall always occurs at the placental site with the exception of a very few cases, where a secondary attachment is formed by the ovum, which is not covered by reflexa, and the rupture then occurs at this point.

The examination of the enlarged Fallopian tube to determine pregnancy should be conducted in the following manner: First, blood clots which are attached to the tube are searched for fragments of chorionic villi, after the manner recommended for the examination of blood clots in the uterus. (Page 221.) If the search is not successful, the placental site, recognized by the fibrin deposits on the serosa, or by marked bulging, or thinning of the tube, must be found, incised, and placental fragments again sought for. If the placental site has been found, the tube may now be opened on the opposite side, otherwise, if the search is not successful, the tube must be opened in the free portion, the clots elevated and at the point where they are adherent to the cavity of the tube is the placental site. Search at this point will usually disclose white fragments of tissue in the blood clot and these must be taken for the fresh examination, after which the loose portions of the clot are removed and the placental site is cut out of the tube and hardened.

M. F., F. M., or celloidin. N. S.

By low power the muscular wall of the tube and the mucous membrane beyond the placental site, are seen to be well preserved. At the edge of the placental site the mucous membrane rises and for a certain distance rests upon the blood clot which includes the ovum. At the serotina the mucous membrane is absent. The muscular coats of the tube are reduced to a few bundles of fibers, in the neighborhood of which are a plentiful supply of dilated vessels. About these vessels may be seen the cause of the remarkable thinning of the tubal wall, namely, masses of fœtal cells, many of which lie in the vessel lumenæ. In early tubal pregnancies the action of the fœtal cells may be seen in a complete infiltration of the muscular coats with masses of syncytium and cells from the layer of Laughans, but as the pregnancy advances, these elements disappear. The blood clot contains a greater or smaller number of chorionic villi. The deposits of fibrin on the serosa opposite the placental side are usually in an advanced state of organization, beneath which occasional clefts are found lined with proliferated serosa epithelium, similar to the gland-like nests found beneath the fibrin deposits in chronic pleuritis.

XVI. THE MALE GENITAL ORGANS.

1. Gonorrhœa.

The principal symptoms of gonorrhœal infection of the urethra is a free purulent discharge. This discharge in the early stages of the infection contains the specific organism of the disease, the gonococcus, which may readily be detected in cover-slip preparations of the pus. (Plate XXVIII., Fig. 4.) The organisms occur in pairs and have the opposing sides somewhat flattened. They occupy the bodies of leucocytes or are gathered together in groups. The organisms are decolorized by Gram's method (stain with Gram, restain with vesuvin). In

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chronic gonorrhœa the organisms are more difficult to find, but should be sought for in the morning drop, or the mucous threads (trippe faden) which appear in the urine after massage of the prostate has been practiced.

2. Hypertrophy of the Prostate.

Enlargement of the prostate may be produced by increase of the muscles and connective tissue, or by hypertrophy and proliferation of the glandular structure.

The structure of both the male and female prostate (glands of Skene) is characterized by the intimate arrangement of muscle fibers and glands. M. F., F. M., N. S.

Sections of the enlarged prostate show a predominating increase of muscle in some places, of glandular structure in others. (Plate XXIX., Figs. 1–2.) The glands of the prostate are lined with a double layer of cylindrical epithelium, which when the glands are dilated, takes on a cubical form. The formation of cysts in the prostate is common, especially in old men, where they may be detected with the naked eye and contain fine brown bodies, which, when plentiful, give the cut surface of the gland an appearance as if snuff had been scattered over it. When the fine brown dust from the cystic glands is examined fresh, it is found to be made up of small spherical or irregular bodies which possess definite concentric layers and occasionally radiating striation. (Plate XXIX., Figs. 1–3.) When treated with iodine the bodies take on a brownish yellow or a blue or violet color. Because of the blue and violet reactions they are known as corpora amylacea. (For special stains see p. 50.) In the microscopic sections these bodies are seen to lie in the lumenæ of the glands, for which reason they are supposed to be formed from altered gland secretion and to be a form of concretion. Corpora amylacea are found in the secretion of the prostate, which may be obtained by massaging the gland, and in the seminal fluid after ejaculation. The seminal fluid contains, beside these bodies and spermatozoa, certain glistening spheres which come from the seminal vesicles, and a large number of double-pointed, octohedral crystals (so-called Böttcher's crystals), which are deposited from the secretion of the prostate.

3. Purulent Prostatitis.

Purulent inflammation of the prostate may occur as a sequel to gonorrhœa, infection following catheterization or purulent cystitis. Macroscopically the prostate presents a yellowish striated and spotted appearance. M. F., F. M., Gram or methylene-blue.

In this case the pus cells are found beneath the epithelium and the adjacent connective tissue is likewise infiltrated. The organisms proliferate in the gland lumenæ and spread through the entire organ. The female prostate is an important factor in the prolongation and persistence of the gonorrhœal process in women.

4. Tuberculosis of the Testicle and Epididymis.

Structure.—The stroma of the testicle consists of fibrous connective tissue which, according to the age, development and nutrition of the organ includes a varying number of plump, spindle-shaped cells, the protoplasm of which is coarsely granular. These are often present in sufficient number to form a more or less definite network in

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the stroma between the tubules (so-called intermediate cells). The granulation of the protoplasm of these cells is due in part to fat and in part to pigment. The tortuous secreting canals, the so-called tubuli contorti, are surrounded by a special connective and elastic tissue structure, which, directly beneath the epithelium is condensed into a membrana propria. The membrana propria is composed almost entirely of elastic fibers (Benda and Perutz). The lumen of the seminal vesicles or tubuli contorti is lined with the multiple layers of the specific epithelium of the organ. The tubuli recti or straight tubules are lined with a single layer of low cylindrical epithelium. These empty into the rete testis which is an irregular cleft lined with simple layers of squamous epithelium. The vasa efferentia which leads from the rete testis has a well-defined connective-tissue wall and the lumen is lined with high, in part ciliated, cylindrical epithelium. The vas deferens is lined with a single layer of ciliated epithelium. The circular muscular coat is supported from without by a longitudinal muscular layer.

The epididymis is more commonly and more severely affected by the tubercular process than is the testicle. Where both are involved the epididymis usually contains large areas of caseation and the testicle a greater or smaller number of tubercles which are usually localized about the hylum and spread out into the tissue disappearing near the periphery. Tuberculosis of the epididymis is usually associated with tuberculosis of the spermatic cord, for which reason in extirpation of the testicle for tuberculosis, the spermatic cord should be carefully examined. The bacilli are transported through the seminal vesicles. M. F., F. M., N. S., tubercle bacilli stain, elastic fiber stain.

In suitably stained sections the bacilli are not infrequently found in the lumenæ of the unaltered seminal vesicles. The irritation produced by the organisms leads to round-celled infiltration and proliferation of the connective-tissue stroma surrounding the vesicles. The nodular form of tuberculosis begins in the wall of the vesicles and for this reason causes rapid and extensive destruction of the elastic fibers which form the basement membrane of the vesicles. This destruction of the elastica seems to be due to a specific toxic action of the organism which is exerted during the stage of granulation tissue formation. If the period of granulation tissue formation is prolonged the destruction of the elastic tissue is complete. When caseation appears early remnants of the elastic structure remain. The destruction of the elastica ceases with the beginning of caseation. In the interstitial form of tuberculosis of the testes the elastica is affected to a much smaller degree probably because the toxic substances elaborated by the bacillus do not come in direct contact with the elastic structures. The extensive and early destruction of the elastic fibers of the testicle is a valuable diagnostic feature and distinguishes the nodular form of tuberculosis from syphilitic gummata of the organ (see Syphilis of the Testicle). We do not know to what extent the specific epithelium takes part in the proliferative changes induced by the organism or whether it is capable of entering into the formation of tuberculous giant cells. As caseation appears the epithelium is destroyed. Beside the tuberculous change within the seminal vesicles, typical epitheloidal tubercles may develop in the stroma of the epididymis and testicle. (Plate XXVIII., Fig. 5.) Tuberculosis of the spermatic cord appears in the form of tuberculous proliferation in the mucous membrane, diffuse caseation in the lumen and tubercles in the stroma.

5. Syphilis of the Testicle.

The testicle is of all organs the most commonly affected by syphilitic change. The tissue of the affected organ either contains fine ribs of fibrous tissue, which prevent the picking up of the seminal vesicles (with forceps) or the substance of the testicle contains single or multiple caseated foci (gummata). Both of these forms of the disease may be present in the same organ.

PLATE XXVIII.

Fig. 1.

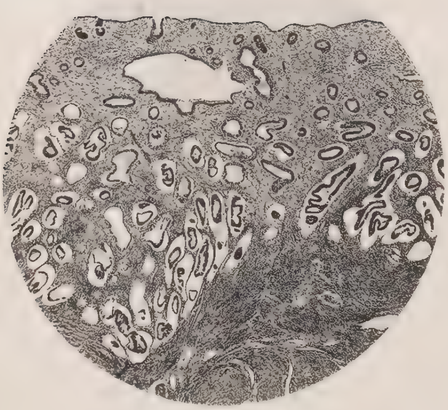


Fig. 2.

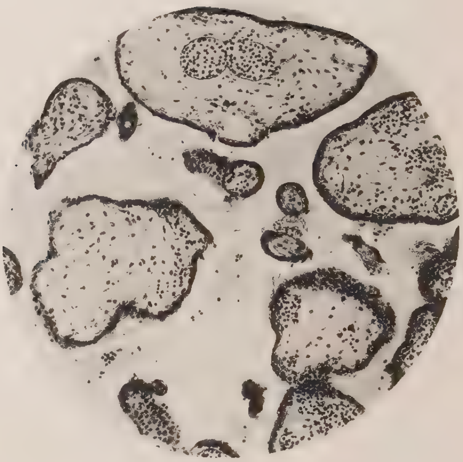


Fig. 3.

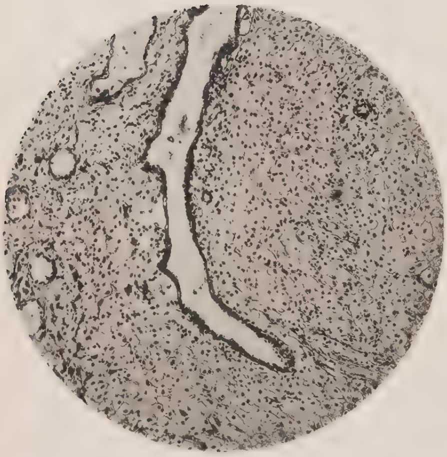


Fig. 4.

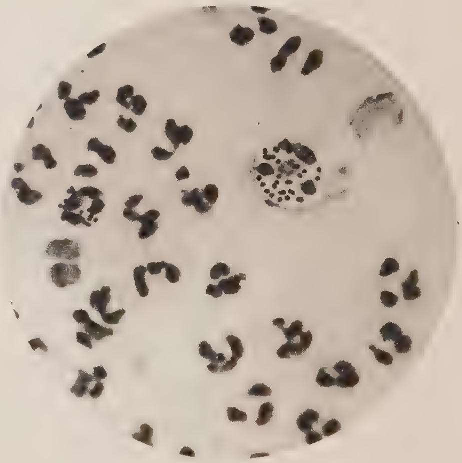


Fig. 5.

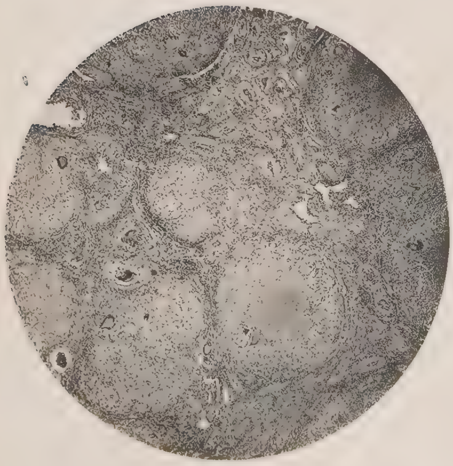
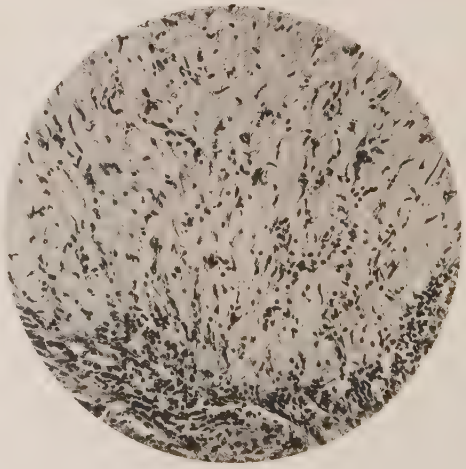


Fig. 6.



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M. F., F. M., N. S., or elastic fiber stain. Under the microscope the connective tissue stroma of the organ is found to be in a more or less active state of proliferation. This may be diffuse or appear in nodular form with beginning central necrosis. These focal lesions are in every respect similar to the gummata found in the liver (Plate XXVIII., Fig. 6), and may be distinguished from tubercles by their size, the large amount of fat which the necrotic material contains (*fresh examination*), the scarcity of giant cells and absence of tubercle bacilli and the almost unaltered condition of the elastic fibers when these are included in the gumma (see Tuberculosis of the Testicle). An examination of the vessels adjoining the diseased tissue will greatly assist in the diagnosis. In syphilis, these, especially the veins, will show a more or less extensive endophlebitis or endarteritis. In the immediate neighborhood of the caseated areas the changes in the vessel walls are sufficient to produce obliteration (see Blood Vessels).

General atrophy of the specific epithelium of the seminal vesicles follows prolonged inflammation of the organ, and occurs physiologically with advancing age. The layers of epithelium become first thin and then disappear. The connective and elastic tissue surrounding the vesicles are affected by hyaline metamorphosis.

XVII. TUMORS.

(Neoplasms.)

All new formations of tissue may be divided into three principal groups, which gradually merge into one another. The differentiation of these types will continue to present difficulties until the cause of the most obscure of all tissue formations and malignant tumors is established.

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M. Simmonds. Die Veränderungen des Hodens bei experim. Verschluss des Samenleiters, Mitteil. aus den Hamburger Staats-Krankenanstalten, 1899.

DESCRIPTION OF PLATE XXVIII.

Fig. 1. Endometritis glandularis cystica. See p. 217. Low power. Hæmatoxylin. Below muscular structure of the uterine wall. Resting upon this the thickened mucous membrane containing sections of uterine glands. These are enlarged and describe a tortuous course. Above beginning cyst formation.

Fig. 2. Chorionic villi. See p. 221. Middle power. Hæmatoxylin. Oblique and transverse sections of placental villi. The double layers of epithelium are not sharply defined (nearly mature villi). The stroma consists of stellate branching connective tissue cells. Above a villus with central vessel containing nucleated red blood corpuscles (magnifier).

Fig. 3. Decidua serotina. See p. 220. Middle power. Hæmatoxylin. At the center a uterine gland. The remaining structure composed of large vesicular pale decidua cells with round or oval, relatively small nuclei. To the left and below chorionic cells with large, irregular, deeply stained nuclei. These lie in the neighborhood of the blood vessels. Similar cells grouped about a small vessel above and to the right (magnifier).

Fig. 4. Gonorrhœal pus. See p. 226. Oil immersion. Methylene-blue. A large number of leucocytes with typical horseshoe and polymorph nuclei. To the right and somewhat above the center a leucocyte containing pairs of gonococci (magnifier).

Fig. 5. Tuberculosis of the testicle. See p. 227. Low power. Hæmatoxylin. Above seminal vesicles in cross section. The rest of the field is composed of closely packed, well-developed tubercles with central caseation. Many of the tubercles contain giant cells (magnifier).

Fig. 6. Periphery of a gumma in the testicle. See p. 227. High power. Hæmatoxylin. Pale granulation tissue, the slender spindle elements penetrating into the necrotic mass.

1. Tissue which is developed to replace lost or disintegrated structures (regeneration). This is effected either by simple repair of the defect (true regeneration), or by enlargement of some other structure which undertakes the function of that which is lost (compensatory hypertrophy). The purpose of the proliferation is the restoration of function.

2. The formation of tissue as the result of inflammatory irritation (inflammatory tumors). The cause of these tissue formations is either direct inflammatory irritation, alteration of anatomical relations or disturbance of function. The purpose of their development is the restoration of function or the protection of the tissue from inflammatory irritation.

3. Neoplasms. Under this heading is usually understood new growths, the cause of whose development we do not understand and whose function is without purpose. It is true that the metastases of primary liver cancer produce bile, but as these structures possess no communication with the surrounding tissues the secretion is of no value to the host. Likewise it has been stated that the cancer cells in cancer of the pancreas are able to carry out the function of the pancreas cells, but this is only a part of their activity and is secondary to their destructive properties. Neoplasms may be defined as useless autonomous growths. (For the views of one of us (Gaylord) on the etiology of cancer see end of chapter.)

The above classification does not enable us to determine the nature of new growths for the reason that it is often impossible to ascertain the amount of the loss which the new formation is to replace, or the character of the inflammatory agent. For this reason attempts have been made to ascribe the formation of tumors to certain physiological and anatomical changes in the tissues. The anatomical or physiological explanation is not satisfactory, as the primary changes in the tissues in beginning tumor formation and the early stages of regeneration or hypertrophy, are, at least in many cases, identical. In making this statement the fact is disclosed that neoplasms are derived from the tissues of the body, that while they are atypical as regards structure and form, the elements which compose them more or less closely resemble some type of tissue cell. All forms of new growths come within the fundamental law of Virchow, "omnis cellula e cellula." The cells of tumors follow the physiological method of cell division, the process of mytosis or karyokinesis, rarely the process of amytosis.

The process of karyokinesis is divided into three phases:

1. **The Prophases.**—(Plate XXXV., Fig. 1.) The nuclear membrane disappears. The chromatin granules are formed into threads which are arranged in the form of a loose skein. The nucleus disappears as a definite structure.

2. **Metaphases.**—(Plate XXXV., Fig. 5.) The chromatin threads (chromasomes) approach the equator of the cell. The central spindle is formed, the polar rays appear (Fig. 5). The chromatin threads in the equator form the so-called monaster. The separate chromatin threads divide in the long axis and, following the course of the central spindle, approach the opposite centrosomes, producing two stellate figures at the respective poles of the central spindle, the so-called diaster (Fig. 3).

3. **Anaphases.**—This is exactly the reverse of the prophases. The threads forming the two stellate figures become transformed into skeins and about these develop nuclei with nuclear membranes. Division of the nuclei is accompanied by division of the cell body (Fig. 2).

As we are unable to formulate a satisfactory definition for neoplastic formations, so are we unable to establish any satisfactory classification. The most useful is the histogenetic. It is well to note that this classification is based upon mature specialized tissues and *without regard to their embryonic derivation.* Under this classification we recognize:

1. Tumors developed from the connective-tissue group, that is, all tissues which serve purposes of support, movement, formation or transportation of the lymph.
    - (*a*) True supporting structures (connective tissue, cartilage, bone and fat).
    - (*b*) The lymphatic tissues, the blood- and lymph-forming tissues.
    - (*c*) The endo- and perithelium.
    - (*d*) The epithelial coverings of the serous membranes.
    - (*e*) Muscle.
    - (*f*) Tumors derived from normally pigmented cells.
    - (*g*) Tumors derived from blood vessels.
2. Tumors derived from nerve tissue. (Neuroglia and ganglion cells.)
3. Tumors derived from true epithelium. Epithelium of the skin, mucous membranes and epithelial glands.
4. Mixed tumors. Dermoids, embryoid tumors, tumors from adrenal rests, tumors derived from the fœtal ectoblast.

All tumors possess in common a vascular connective-tissue stroma in which the specific tumor cells are embedded. Tumors which contain cells belonging to both the epithelial and connective-tissue group are known as mixed tumors.

It is customary to speak of malignant and non-malignant tumors. The former are so called because the new growth is not limited by the physiological boundaries of the tissue from which it is derived. They likewise produce metastases either by contact or transportation of infected cells through the blood vessels or lymph spaces. A further distinction from the non-malignant form is the frequency with which they are affected by retrograde metamorphosis, *i. e.*, fatty degeneration, calcification, necrosis and the destruction by hemorrhages. These changes may be recognized macroscopically by the yellow (fat), red (blood), brown (pigment), white (calcification), areas of coloration and discoloration (necrosis), which the cut surfaces of these tumors exhibit.

For the investigation of tumors the old rule that the examination of hardened material must be preceded by a similar examination of the fresh tissue, is especially important. The examination should begin by a careful determination, by inspection, of the extent of the tumor. The richly cellular forms of malignant tumors may be recognized by their whitish appearance, which sharply defines them from the surrounding structures. A careful search must be made for suspicious foci in the surrounding tissues, which may be revealed by the sense of touch, as most tumors are denser than the surrounding tissues. In epithelioma of the skin, if the macroscopic examination discloses the fact that the tumor penetrates into the muscular structures, and if the examination of fresh teased particles shows numbers of the tumor cells between the muscle fibers, a diagnosis of malignancy may be made at once. When the macroscopic examination has determined the extent of the tumor, and the character of the tumor cells, as well as the retrograde changes which they have undergone, have been determined by means of teased particles and scrapings, or fresh sections, a portion of the periphery of the tumor should be chosen, as well as any suspicious foci or lymph nodes, and suitable blocks of tissue should be removed for hardening and further investigation.

1. Fibroma.

As the name indicates this tumor is made up of fibrous tissue. The characteristics of the fibromata are as variable as those of the normal connective tissue structures. We distinguish two varieties, the soft and the hard fibromata.

(*a*) **Soft Fibroma.**—These occur most frequently as polypoid growths on the mucous membranes, or as pedunculated nodes upon the skin. The substance of the soft fibroma or mucous polyp, when incised, appears pale and transparent or, when more vascular, of a reddish tinge. By means of a *fresh teased preparation*, the polyp may be broken up into fine fibrillæ and large branching cells. On the addition of acetic acid these are found to contain large vesicular nuclei.

M. F., F. M., N. S., van Gieson or Mallory's connective-tissue stain. The examination of hardened sections shows the substance of the soft fibroma to be composed of large, irregularly shaped cells. The bodies of these cells are rich in protoplasm and are either stellate, pyramidal or spindle-shaped. The prolongations of the cell end in fine fibrillæ, which apparently bind the widely separated cells together. The larger spaces between the fibrillæ and cells appear to be empty. Here and there a larger or smaller round cell may be seen, some of which contain polymorphnuclei, while others are of the round mononuclear type. The vessels in the tumor are widely dilated and their walls unusually thin. Fresh hemorrhages are commonly present or the remains of old hemorrhages may be found in hæmatogenous pigment deposits in the bodies of the cells. The interstices between the network are filled with an œdema fluid which contains so small an amount of albumin that during the process of hardening no precipitate is formed. Occasionally the amount of albumin is sufficient, and a fine granular precipitate fills the open spaces of the tumor.

A second form of soft fibroma is that in which the substance of the tumor is made up of round connective-tissue cells which send out fine fibrillæ in all directions. The nuclei of these cells are likewise round and almost completely fill out the cell bodies. The fine fibrillæ are intimately interwoven and the network contains a certain number of round cells, a large number of mast cells and an occasional lymphocyte.

A third form (Pl. XXIX., Fig. 4) is composed of bundles of loose fibrous tissue, the fibrillæ are long and thin and describe a wavy course, running in all directions. Between the fibrillæ are a few very thin and long connective-tissue cells, which end in fibrillated projections. In the interstices of the tissue are found the above-mentioned forms of cells.

(*b*) **Hard Fibroma.**—The ordinary hard fibromata, such as occur in the abdominal wall, the dura mater, etc., present on section a grayish white appearance with irregular elevations and coarse grain. The bundles of fibers run in all directions, and are occasionally arranged about centers forming large nodules. Fresh teased preparations are prepared with difficulty owing to the density and tenacity of the tissue, which, when broken up, consists of coarse fibers within which, after treatment with acetic acid, elongated nuclei may be demonstrated.

M. F., F. M., N.S., van Gieson or Mallory's connective tissue stain.

Under low power a large amount of fibrillated intercellular substance may be detected. Under high power the single fibers of the tissue are found to be much thicker than in soft fibroma, or are so intimately pressed together that they are scarcely distinguishable from one another. In some cases the fibers coalesce to form wide hyaline bands. The crevices between the bands and fibers contain connective-tissue cells, the nuclei of which are long and deeply stained and almost completely fill out the cell body. In other forms of hard fibroma the density of the tissue is produced by a close arrangement of the cells more than by an increase in the fibrillated intercellular substance. In this form the coarse fibrillated appearance of the surface is produced by bands of closely packed shorter or longer spindle cells, and a more or less well-developed intercellular substance. Under high power these cells are found to resemble in size and appearance ordinary connective-tissue cells in various stages of development. The nuclei are elongated or slightly oval. The ordinary fibromata seldom contain elastic fibers and in some forms (keloid), elastic fibers are totally absent.

2. Myxoma.

Myxomata are rarely found as independent tumors but are usually associated with cartilage formation as myxochondroma, or with malignant connective tissue as myxosarcoma. Myxomatous tissue possesses a glassy, transparent character and contains a tenacious, slimy fluid. The character of this fluid may be determined with fresh material by treating it with acetic acid when it is transformed into a thready mass. The thready appearance is particularly characteristic at the edges of the material. A small portion of the tumor should be crushed between a cover-glass and slide, when a large number of branching cells with fine prolongations and finely granulated protoplasm will be seen in the mucoid substance.

For the preparation of hardened material, sublimate, alcohol, and Mueller-formaline may be chosen, depending upon the special methods for the demonstration of mucus, which are to be employed. The protoplasmatic projections of the cells may be demonstrated by hardening in alcohol, embedding and staining according to Beneke's modification of Weigert's fibrin stain.

For ordinary investigation M. F., F. M., N. S.

In sections from hardened material the mucoid substance is supported by a more or less well-developed network formed by the long protoplasmatic projections from the stellate cells which compose the tumor. The whole presents an appearance much like œdematous connective tissue. The mucoid material fills in the interstices between the cells and cell projections, and is usually homogeneous in character. Here and there a few round cells may be seen. The mucoid material may be demonstrated by special methods, being stained either red or violet according to the method employed, and usually presents the thready appearance noted after the treatment of fresh material with acetic acid. The cells in such preparations are usually colorless, the nuclei dark blue.

3. Psammoma.

Tumors of the dura mater, whether fibromata, endotheliomata, or sarcomata frequently contain deposits of chalk. Unless the tumor is totally calcified, when it cannot be cut, the deposit of chalk usually takes the form of coarse granules, giving to the cut surface a sandy appearance. The non-malignant form of sand-containing tumor is usually a fibroma (psammoma). The external characteristics of these tumors are very much like the ordinary fibrous connective-tissue tumors.

The *fresh examination* discloses the calcified bodies as dark spherical structures surrounded by dark rings. When treated with hydrochloric acid the dark center of the sand body disappears and fine bubbles of gas are liberated (CO_2). Beside the spherical forms of chalk deposit the tumor commonly contains spindle-shaped accumulations of fine chalk granules (high power).

M. F. or F. A. Decalcify. F. M. or embed. N. S. or van Gieson or Mallory's connective tissue stain.

In microscopic appearance the structure of the tumor resembles that of an ordinary hard fibroma. Here and there the cells possess a characteristic arrangement in concentric layers forming spherical bodies usually arranged along the blood vessels. The centers of these nodes frequently show evidence of beginning hyaline degeneration, while about the peripheries the staining qualities of the nuclei are preserved. It is in these outer layers that the deposit of chalk is greatest. If the preparation has been insufficiently decalcified the peripheral calcified portion stains deeply with hæmatoxylin, or with van Gieson takes on a bright yellow, while the hyaline centers are red. The tumor frequently contains spindle-shaped spicules of chalk. These are calcified hyaline bundles of connective tissue.

4. Lipoma.

The macroscopic diagnosis of fat tumors presents no difficulties because of the similarity in appearance of the tissue of the tumor and ordinary fat tissue. The tumor usually possesses a lobular structure.

M. F. Celloidin or paraffine. N. S.

When *examined fresh* (shear section) or in hardened sections the characteristics of the tumor are those of ordinary fat tissue. In hardened material the fat is usually extracted during the process of hardening and the fat cells are represented by spherical openings. Where the tumor contains any considerable amount of connective tissue we speak of a fibro-lipoma.

5. Myoma.

The rarest forms of muscle tumor, those composed of striated muscle fibers (rhabdomyoma) are almost all of congenital origin and are found in the genito-urinary tract. Many of these tumors possess a more complicated structure and will be considered in the chapter on teratoma. The striated muscle fibers, which occasionally form a considerable portion of the tumor, may be detected in fresh teased preparations. These (likewise in hardened sections) (M. F., F. M., N. S., or van Gieson) show marked variations from the normal form and structure of mature striated muscle fibers and are more closely related in appearance to certain stages in the embryonic development of striated muscle. The elements isolated from the tumor may be spindle-shaped with a mere suggestion of transverse striation, and when this form predominates, a diagnosis of myosarcoma may be made. Beside the spindle elements curious cylindrical structures may be found with a peripheral transversely striated sarcolemma and a pale sarcoplasm with the nuclei arranged in rows. Some of the cells are stellate in character and send out more or less extensive processes from the cell body.

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DESCRIPTION OF PLATE XXIX.

FIG. 1. Glandular hypertrophy of the prostate. See p. 227. Middle power. Hæmatoxylin. Irregularly shaped glandular cavities lined with columnar epithelium. In the gland cavity to the right a laminated concretion (magnifier).

FIG. 2. Muscular hypertrophy of the prostate. See p. 227. Middle power. Hæmatoxylin. Atrophic glandular openings filled with concretions and surrounded by muscular structure with long, slender nuclei (magnifier).

FIG. 3. Corpora amylacea. See p. 227. High power. Hæmatoxylin. The concentric striation is plainly visible. The straight lines across the concretion are produced by imperfections in the knife.

FIG. 4. Fibroma. See p. 232. Middle power. Hæmatoxylin. Tumor is made up of fibrous connective tissue with but few nuclei. The wavy, varying direction of the fibers plainly visible.

FIG. 5. Myoma. See p. 234. Middle power. Hæmatoxylin. Tumor consists of bundles of muscle having a general transverse direction. The nuclei of the cells are rod-like and slender. To the right a small vessel (magnifier).

FIG. 6. Spindle-cell sarcoma. See p. 236. Middle power. Irregularly arranged, closely packed groups of spindle cells with oval or short and spindle-shaped nuclei. The arrangement of the cells about certain centers should be noted.

PLATE XXIX.

Fig. 1.

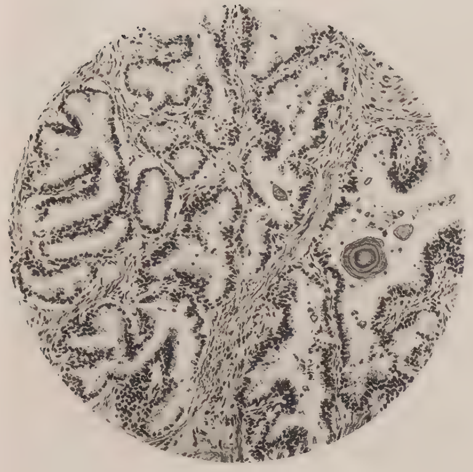


Fig. 2.

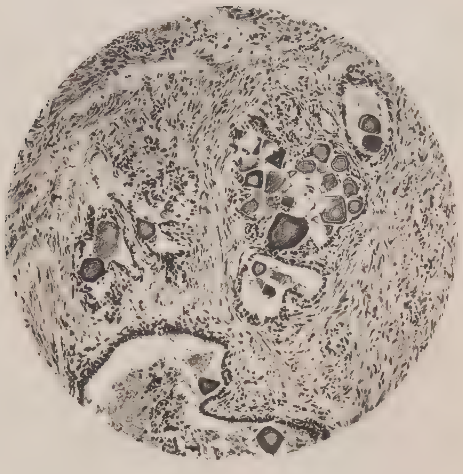


Fig. 3.

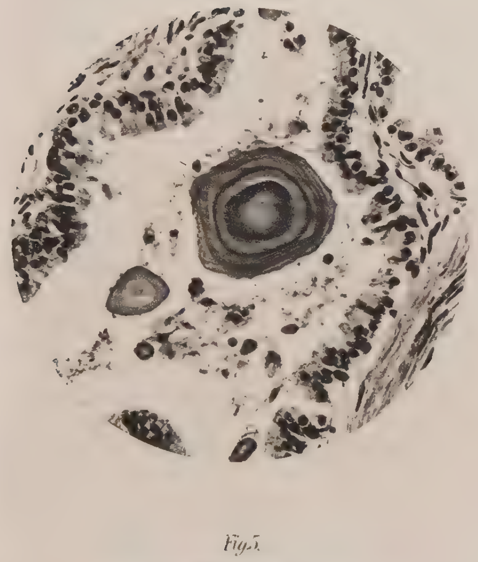


Fig. 4.

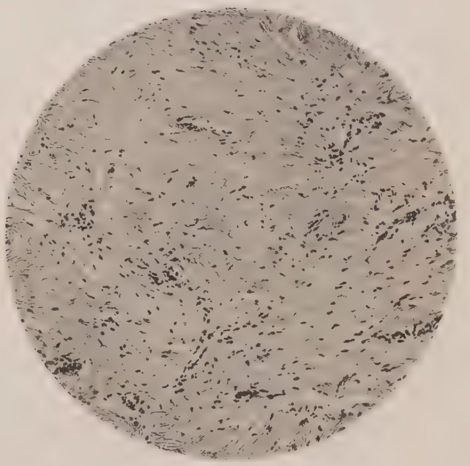


Fig. 5.

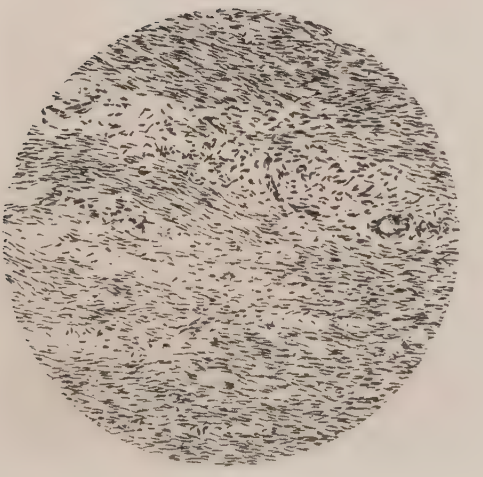
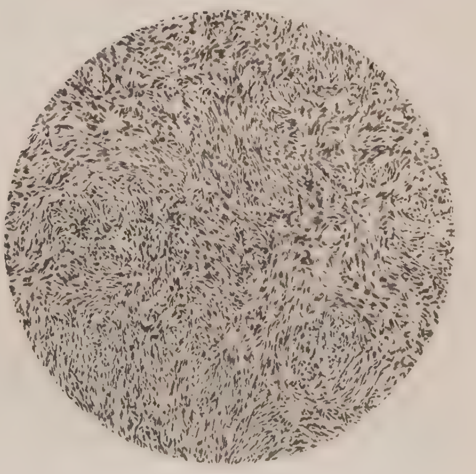


Fig. 6.



These processes may show cross and longitudinal striation. Where the transverse striation is absent or obscured by a well-defined longitudinal striation these elements might readily be mistaken for ganglion cells. In the fresh state a considerable amount of glycogen may be demonstrated in the fibers by appropriate treatment with iodine solution. Where the glycogen is to be demonstrated in sections the material must be hardened in alcohol.

A tumor which is more common than rhabdomyoma is the leiomyoma, composed of smooth muscle fibers, most frequently found in the uterus where the growths take the form of more or less irregular spheres. The cut surfaces of these tumors present elevations and depressions due to the irregular contraction of the muscular structure and the crossing and interspersing of the muscle fibers. The color is dependent upon the amount of blood which the tumor contains but is occasionally altered by degenerative processes (fatty degeneration, pigmentation, etc.). The presence of fat may be determined in *fresh teased* preparations. If the form and dimensions of the muscle fibers are to be ascertained a piece of the myoma must be macerated in potassium hydrate. Sections of hardened material are best suited for purposes of orientation.

M. F., F. M., N. S. or van Gieson or Mallory's connective-tissue stain. Under low power the structure of the tumor somewhat resembles that of a fibroma in that groups of cells run in the same direction, and these bands or groups are constantly crossing or intermingling. Under high power the cells are found to differ from those of fibroma in their slender form and long rod-like nuclei. (Plate XXIX., Fig. 5.) The rounded ends of the nuclei and the parallel arrangement of the cells distinguish the smooth muscle fibers from the more oval or pointed nuclei and wave-like arrangement of the cells in fibroma. This differentiation, however, does not suffice for all cases and it occasionally happens that it is impossible to determine whether a muscle or a connective-tissue cell is under observation. If a section of myoma be stained after van Gieson the muscle fibers take on a yellowish brown coloration and the surrounding connective-tissue structure a brilliant red. Cross sections of the muscle fibers produce structures which closely resemble round cells, oblique sections produce oval forms. Where a transverse section includes a complete muscle bundle, a group of pseudo round cells surrounded and supported by a fine connective-tissue stroma is produced. As the nuclei of the muscle fibers do not all lie in the same plane, it is obvious that in a cross section a certain number of the cells will appear as if they possessed no nuclei. If the bundles of muscle fibers are more or less continuous and the intermediate connective tissue is well developed the tumor should be known as a fibromyoma. The connective tissue stroma is commonly affected by hyaline degeneration. Beside the more or less sharply defined spherical myomata a more infiltrating form of tumor is found in the uterus, usually about the openings of the Fallopian tubes. These not uncommonly contain glandular structures which rest upon, and are bounded by, the muscle fibers. The cells of these gland-like structures are either spindle-shaped or round and are highly suggestive in structure of the mucous membrane of the embryonic uterus. These inclusions are in part remains of the Wolffian body and in part isolated pro-

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jections from the duct of Müller. The existence of these tumors indicates rather strongly the derivation of many myomata from embryonic rests.

Occasionally myomata exhibit an unusually rapid growth. On section the tumor appears more white than grayish red. In microscopic sections it is composed of closely packed short oval and spindle cells. So long as these cells are uniform in form and size, a diagnosis of malignancy should not be made, as the cells do not differ from those of embryonic muscle fibers. If, however, the cells and nuclei vary in form and size, especially if many of them are round, and more or less frequent giant cells are found, the tumor is sarcomatous. Here and there more or less well-preserved muscle fibers may be found, which indicates that the tumor has resulted from sarcomatous change in fibro-myoma. In many cases it is difficult or impossible to determine whether the cells of the tumor are transformed muscle fibers (malignant myoma), or connective tissue cells (sarcomatous invasion of a myoma).

6. Sarcoma.

All malignant tumors which are derived from the cells of the connective-tissue stroma, from bone or cartilage, from the lymph spaces and the coverings of the blood vessels are included under the term sarcoma. These are divided according to the form and character of the cells:

(*a*) **Spindle-cell Sarcoma.**—In macroscopic appearance a sarcoma resembles more or less closely an ordinary hard fibroma. The greater the rapidity of growth and the greater the number of cells in the tumor, the more the color approaches white. Brown spots in the tumor indicate pigmentation, yellow coloration, fat, and dirty discoloration of the tissue is due to necrosis. The more malignant the tumor, the more rapid its growth, the more generally it is affected by degenerative processes. The cells of the sarcoma may be satisfactorily studied in *fresh teased* preparations. The isolated elements are spindle-shaped with more or less well-developed protoplasmatic projections which frequently branch. Occasionally the cells are not uniform and the large spindle elements are mixed with cells of irregular shape or with large round elements which may or may not possess protoplasmatic processes. During the process of teasing the cells are commonly broken up and free nuclei appear in the embedding fluid. In some cases scarcely a well-preserved cell will be found, the entire preparation consisting of cell detritus and free nuclei. The liberated nuclei may be distinguished from the complete cells by their highly refractive nuclear membrane which gives them a sharply defined contour, whereas the cell protoplasm of the complete cell possesses an indefinite and irregular periphery. On the addition of acetic acid the nuclei of the unbroken cells appear sharply defined. These show more or less deviation from the prevailing oval form and will be further considered in the investigation of stained sections.

M. F., F. M. or van Gieson, or Mallory's connective-tissue stain.

When investigated in sections the structure of the spindle-cell sarcoma is seen to consist of closely packed spindle or shorter oval elements. Where the latter form of cells predominates, these are, as a rule not so closely arranged, and a delicate fibrous intercellular structure may be made out between them. (Plate XXIX., Fig. 6.) (The relation of the cells of the sarcoma to its blood vessels will be considered under Angiosarcoma.) Usually the amount of intercellular substance is in proportion to the density of the tumor but many exceptions to this rule will be found. Those which contain a sufficient amount of intercellular substance are classed as fibro-sarcoma. It is often difficult to determine whether a tumor belongs to the non-malignant fibro-sarcoma group or to the malignant spindle-cell type. When the cells of the tumor are unusually large and the nuclei show deviations from the normal form of the connective-tissue type (polymorph cellular sarcoma), the malignancy of the growth is assured. The nuclei are unusually large, contain a large amount of chromatin substance, are round, oval, or show various forms of distortion (indented, horseshoe or branching) or are split up into various forms (fragmentation). The distribution of the chromatin in the nucleus is very irregular. It may all be deposited directly beneath the nuclear membrane, accumulated in a thickened network, or when degeneration has affected the nucleus, the chromatin may take on a characteristic rosette-like arrangement; or may be dissolved in the nuclear fluid when the entire nucleus stains diffusely. Beside deviations from the normal in the distribution of the chromatin substance, the nuclei which are undergoing division show many atypical karyokinetic figures. These are of the greatest variety, the deformity being inequality of size in the double asters, or practical non-development of one of the asters. Beside simple asymmetry the nuclei may be divided into three, four or even six parts, in which case the lines of union of the centrosomes form irregular geometrical figures, triangles, tetrahedral and octahedral forms. The protoplasm of the cells is affected more or less extensively by hyaline, colloid, mucoid and hydropical degeneration. Occasionally migratory cells will be found included within the protoplasm of the tumor cells. A certain number of these inclusions are exceedingly suggestive of possible parasites. The cells of the tumor show no attempt at uniform arrangement except about the thin-walled blood vessels where they occasionally form a supporting structure with the cells running parallel to the vessel. Round-celled infiltration is usually found about the blood vessels.

(*b*) **Round-cell Sarcoma.**—Round-cell sarcoma is usually softer than the other forms of sarcoma and on section is somewhat whiter. On *fresh examination* the entire structure of the tumor is composed of round cells, more or less uniform in size in the same tumor, but with occasional variation. Some of these tumors are composed of small round cells closely resembling lymphocytes, others of spherical elements of two or three times the diameter of a white blood corpuscle, so that it is customary to speak of small round-cell sarcoma and large round-cell sarcoma. The suggestion of a stroma is here and there found in fine intercellular fibrillæ. The topography of the tissue can only be ascertained in hardened sections.

M. F., F. M., N. S., Mallory's connective-tissue stain.

The cells of the tumor show a more or less definite structure in rows or spherical nests which are separated from each other by a vascular connective-tissue stroma. In large round-cell sarcoma the connective tissue stroma may be so well developed and the nests of cells so definitely separated as to warrant the term *alveolar sarcoma.* This division of the cells into alveoli is, however, not so sharply defined when examined under high power, when it is seen that the stroma sends out fine projections between the cells. The small round-cell sarcomata are commonly devoid of well-defined stroma and are supported by a system of fine capillaries and an exceedingly delicate reticulated network. (Plate XXX., Fig. 1.) The more definite the reticulum and the smaller the type of cells the more the tumor resembles ordinary lymphatic tissue with the exception that the characteristic structure of the lymph nodes is wanting. This type of tumor is usually derived from lymphatic tissue (lymph nodes of neck, intestinal tract, etc.), and is known as lympho-sarcomata. The tumor begins by enlargement of a lymph node. The lymphatic structure rapidly penetrates and breaks through the capsule of the node and invades the surrounding tissue including the neighboring lymph nodes. Metastases in widely distant organs are rare. The lympho-sarcomata are closely allied in appearance to the enlargement of the lymph nodes affecting entire systems in the course of leucæmia and Hodgkin's disease. Enlargement of the lymph nodes in these cases is possibly of an infectious nature but the lymphatic structure never shows a tendency to widespread invasion of the surrounding structures. In the early stages of lymphatic leucæmia the tissue of the enlarged lymph nodes is so infiltrated with lymphocytes that the characteristic structure of the node is obliterated. In the later stages of the disease proliferative changes affect the connective-tissue stroma and produce what are known as hard lymphomata. Many cases of non-leucæmic multiple lymphomata are associated with tuberculosis: at least the enlarged lymph nodes contain typical tubercular structures. Ordinary round-cell sarcoma is easily distinguished from lymphosarcoma and lymphoma, which possess a more or less well-defined reticulum. With our present means it is impossible to draw a sharp dividing line between these different forms of sarcoma. Spindle-cell sarcoma developing in the connective-tissue stroma of the lymph nodes may be readily differentiated from any of the various forms of lympho-sarcoma.

(*c*) **Giant-cell Sarcoma.**—Giant-cell sarcoma usually develops in some portion of the osseous system. The *fresh examination* discloses the presence of characteristic multinuclear giant cells.

M. F., F. M., N. S. or Zenker. Embed. Mallory's connective-tissue stain.

It has been stated that ordinary sarcoma, especially of the large-celled variety, contains a certain number of multinuclear cells with unusually large cell bodies. In these forms of sarcoma, however, all degrees of variation from the predominating type of the tumor to the multinuclear cells may be traced. Under giant-cell sarcoma is usually understood a form of sarcoma in which a certain number of the cells are unusually large, and sharply defined against the background of tumor structure, which usually consists of spindle sarcoma cells. These giant cells may be from 20 to 30 times as large as the spindle cells of the tumor and are recognized by the unusual number of small, oval nuclei which they contain. (Plate XXX., Fig. 3.) The peripheries of these cells are frequently extended into one or more definite protoplasmatic prolongations or processes, which are lost in the surrounding tissue. The nuclei are generally grouped together near the center, which distinguishes them from the typical giant cells of tuberculosis where the nuclei are usually in crescentic arrangement near the periphery. The central arrangement of the nuclei is the only similarity which the giant cells of sarcoma show to the osteoclasts or giant cells of the bone marrow, which possess a single budding nucleus. Sarcomata which are derived from the bone marrow usually contain a large number of sarcomatous giant cells which are scattered through the tumor structure in irregular groups. A special form of tumor which is derived from the periosteum of the inferior maxillary bone and usually develops in the alveolar process, the so-called epulis, must be distinguished from malignant giant-cell sarcoma. These tumors are usually non-malignant, are about the size of peas or hazelnuts and on section are of a more or less well-defined brown color. On microscopic section the tumor is composed of fibro-sarcomatous tissue which includes a large number of giant cells more or less regularly distributed. This regular arrangement of the giant cells and the large amount of fibrous groundwork distinguish these tumors from the malignant giant-cell sarcomata. (Plate XXX., Fig. 2.)

REFERENCE:

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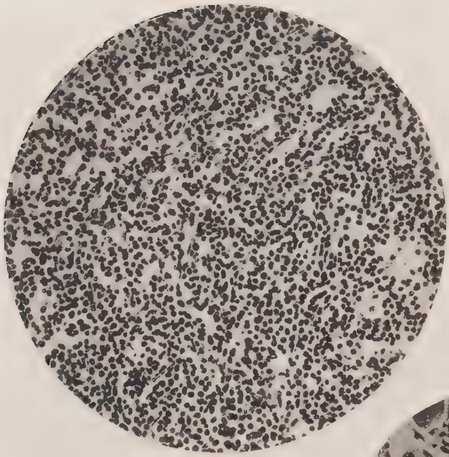
PLATE XXX.

Fig. 1.



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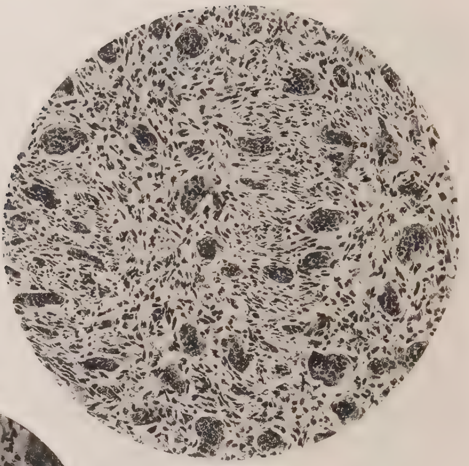

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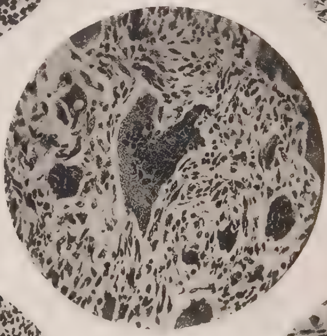

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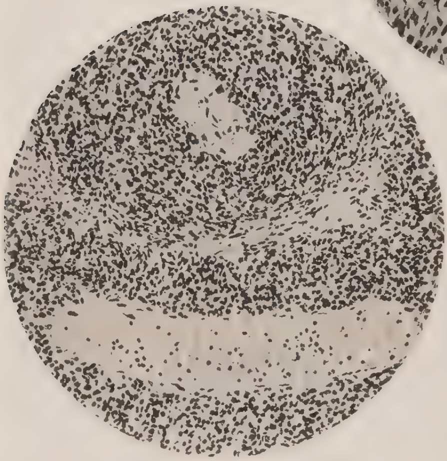

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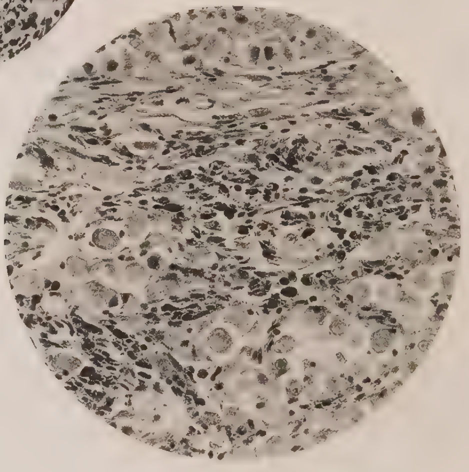

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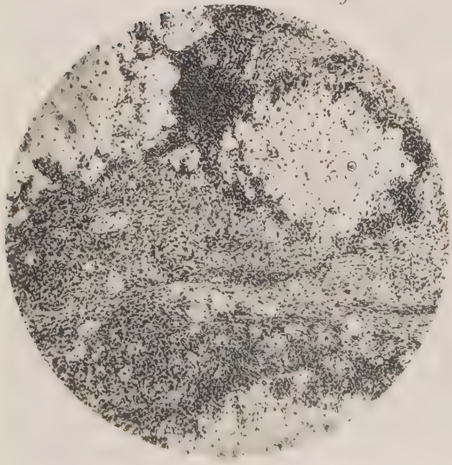
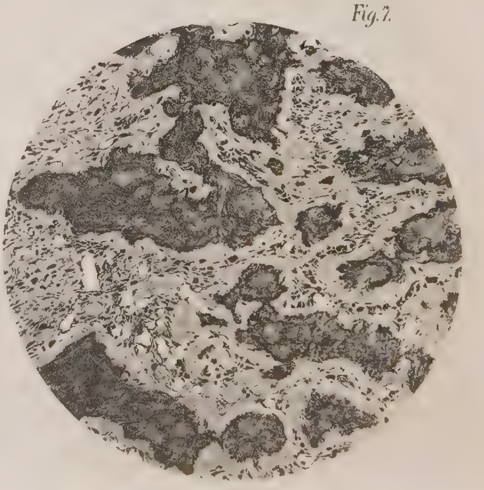


Fig. 7.



(*d*) **Osteofibroma** (**Osteosarcoma**).—The connective tissue or sarcomatous tumors of the osseous system are usually distinguished by their great density and hardness, which is due to more or less well-developed osseous structures, which may be recognized macroscopically (osteofibromata, osteosarcomata).

M. F. Decalcify. F. M. or embed. N. S. or van Gieson or picrocarmine or celloidin. Schmorl's method for the demonstration of bone corpuscles.

Sections of an osteofibroma may be recognized even under low power by the irregularly formed bone trabeculæ. Under high power the transformation of the cells of the tumor into typical bone-forming cells may be traced. These deposit osteoid material which includes more or less atypical bone corpuscles. The newly formed trabeculæ are usually free from chalk deposit (recognized by the staining qualities), in which case the tumor is spoken of as an osteoid fibroma or osteoid sarcoma, as the case may be. The differentiation of the osteoid trabeculæ and islands of osteoid substance from the surrounding tissue is never so definite in these tumors as during the steps of normal bone formation. When the osteoid trabeculæ become calcified we speak of osteofibroma or osteosarcoma. (Plate XXX., Fig. 7.) The growth of the trabeculæ may proceed as in normal bone formation by the apposition of osteoblasts and the deposition of osteoid substance. The disintegrators of the bone structure, the osteoclasts, may likewise be encountered. From what has been stated it will be readily seen that a differentiation between inflammatory bone formation and osteofibroma and sarcoma may in special cases be exceedingly difficult. Sarcomata which are derived from the periosteum or medulla of the bone may become calcified. In these cases the chalk is deposited in an irregularly formed hyaline groundwork without the formation of trabeculæ or bone corpuscles.

(*e*) **Chondrosarcoma.**—Cartilaginous formations in a sarcoma may be recognized macroscopically by the bluish white character of the deposits. M. F., F. M., methylene-blue or stain with lithium carmine and short methylene-blue stain. Or hæmatoxylin alizarin (1-per-cent. aqueous solution). In sections stained with methylene-blue the deposits of cartilage appear as dark blue islands in the sarcomatous tissue. In preparations stained with hæmatoxylin alizarin the cartilage is usually a brilliant orange with a bluish center. (Plate

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DESCRIPTION OF PLATE XXX.

Fig. 1. Small round-cell sarcoma. See p. 237. Middle power. Hæmatoxylin.

Fig. 2. Epulis. (Giant-cell sarcoma.) See p. 238. Middle power. Hæmatoxylin. Large multinuclear giant cells more or less irregularly distributed through the structure of spindle cells (magnifier).

Fig. 3. Multinuclear giant cell from Fig. 2. High power. Hæmatoxylin.

Fig. 4. Angiosarcoma. See p. 241. Middle power. Hæmatoxylin. Above a vessel in cross section. Below one in transverse section. Both are surrounded by a regularly arranged covering of sarcoma cells. The endothelium of the blood vessels plainly discernible (magnifier).

Fig. 5. Melanoma. See p. 243. High power. Alum carmine. The cells of the tumor are mostly spherical and free from pigment. These show more or less well-defined alveolar arrangement (alveolar sarcoma). Within and between the spindle cells of the stroma are granules of pigment.

Fig. 6. Chondrosarcoma. See p. 240. Low power. Hæmatoxylin alizarin. Above two, below one island of cartilage in which the cartilage cells may be plainly discerned (magnifier). These are surrounded by round, oval and spindle-shaped sarcoma cells. Near the center definite spindle-cell structure with small islands of cartilage.

Fig. 7. Osteofibroma. See p. 239. Middle power. Hæmatoxylin alizarin. Deeply stained and irregularly formed bone trabeculæ surrounded by unstained osteoid material. Between these spindle-cell connective-tissue structure with slender nuclei.

XXX., Fig. 6.) The deposits are intimately surrounded by sarcoma cells and about the periphery the various stages of transformation of tumor cells into cartilage cells may be followed. The sarcomatous tissue is frequently affected by mucoid degeneration or the groundwork of the cartilage may take on a similar change, in which case we speak of a myxochondrosarcoma. If the tissue be hardened in alcohol and stained with polychrome-methylene-blue the hyaline groundwork is stained a deep blue and the coagulated mucoid material red. Where the cartilage is affected by mucoid degeneration the capsules of the cell cavities in the cartilage disappear, for which reason the cartilage cells and their prolongations are unusually well defined against the hyaline groundwork. Cartilaginous deposits may become calcified or more rarely undergo ossification.

(*f*) **Endothelioma (Endothelial Sarcoma).**—Under this term are included tumors derived from the endothelium lining the lymph spaces. They usually develop beneath the skin, especially in the region of the face, parotid gland and the genitals. M. F., F. M., N. S.

The microscopic structure of these tumors is exceedingly variable, the principal distinguishing feature being that the endothelium of the lymph spaces is transformed into cubical, cylindrical or spindle-shaped cells which compose the tumor. Where the tumor is well developed these cells possess a peculiar net-like arrangement, or a more glandular formation, or are arranged in the form of solid cylinders of cells. (Plate XXXI., Fig. 5.) From the description already given it will be seen that it is impossible, at least by the investigation of a single section, to determine whether the tumor is an endothelioma or a carcinoma which has invaded the lymph spaces. This can only be determined when it is possible to trace the actual transformation of the endothelium of the lymph spaces into epithelial-like cells. The endothelium of the lymph vessels at some distance from the tumor presents the usual appearance. As we approach toward the margin of the tumor, the endothelium of the lymph space is found to swell and increase in size and gradually approaches in form and appearance the cells of the tumor. The differential diagnosis of these cases must rest upon the following factors :

1. The net-like arrangement of the cells which follows more or less characteristically the arrangement and distribution of the lymph spaces. A similar picture, however, is produced by the diffuse infiltration of the lymph spaces by carcinoma cells.

2. The location of the tumor in organs which do not normally contain epithelium, as the lymph nodes, bone marrow, etc. In these cases metastatic deposits of carcinoma must be properly excluded. Embryonic inclusions of epithelium, as in the formation of the teeth in the jaw or traumatic displacement of the epithelium, must be likewise excluded.

3. The form of the cells in endothelioma may be cylindrical or may simulate squamous epithelium with pseudo-pearl formation. These never produce any characteristic secretions, hyaline and colloid substance being the only excretory products within the tumor. When the cells simulate squamous epithelium the fine protoplasmatic projections which characterize true epithelium are absent. These distinctions are, however, not infallible, as there are certain epithelial glands which produce hyaline or colloid secretions (prostate, thyroid), and further investigation is necessary to determine how long the characteristic fibrillated projections of the protoplasm persist in the cells of squamous epithelioma (prickle cells).

4. The derivation of the cells when this can be determined.

(*g*) **Angiosarcoma.**—This form of sarcoma develops from the adventitial coverings of the blood vessels and is characterized by its great vascularity, frequent occurrence of hemorrhage and irregular areas of pigmentation. A sharp line cannot be drawn between angiosarcoma and the usual forms of sarcoma. On separating the constituents of the tumor for the *fresh examination* it is found to have a coarse, thready structure and may be separated into coarse filaments which under the microscope contain in their axes blood vessels filled with blood, surrounded by a cylinder of tumor cells. M. F., F. M. or celloidin. N. S. or Zenker, celloidin, Mallory's connective-tissue stain. In microscopic sections the principal characteristic of the tumor is found in a large number of dilated capillaries which appear in longitudinal, oblique and transverse sections. These are surrounded by a thick covering of round or spindle-shaped cells. (Plate XXX., Fig. 4.) The arrangement of these spindle cells is more or less characteristic as they appear to spread outward from the vessel wall in a more or less well-defined radiating arrangement. The engorgement of the thin-walled capillaries is often followed by hemorrhage, which accounts for the frequent pigmentation.

Under the heading of angiosarcoma is included a special form of vascular tumor, the cells of which are derived from the so-called perithelium of the capillaries. The perithelial cells are flattened elements which are attached to the outside of the capillaries and separate them from the surrounding lymph spaces. They are not always demonstrable. The tumors which develop from them, the so-called peritheliomata, are extremely vascular structures most frequent in the kidney, the bones and the skin. When incised the cut surface presents a very characteristic brilliant mottling of red, red-brown, yellow and white. The red and reddish-brown colorations are due respectively to blood and deposits of pigment, both of which may be detected by the fresh examination. The yellow is due to a widespread, high grade fatty change in the tumor cells. If a fragment of the white portion of the tumor be teased in salt solution, the cells which compose it are found to contain highly refractive spherical bodies. When treated with iodine these stain a deep brown and may be recognized as glycogen. The detection of the glycogen in hardened sections is only possible when the tissue has been hardened in alcohol. If a fragment of tissue be treated with sputum the enzyme which it contains will break up the glycogen, which disappears from the tissue.

For the orientation of the tumor celloidin sections are necessary. These may be stained with the ordinary nuclear stains, or Mallory's connective tissue stain. Under the microscope the structure of the tissue is highly suggestive of that of an epithelial gland, for it is composed of well-defined alveoli lined with high cylindrical epithelium and supported by a delicate vascular stroma. Only the larger vessels possess any connective-tissue supporting structure, the walls of the capillaries being in direct apposition with the high cylindrical epithelium which lines the alveoli. These apparently rest directly upon the endothelium of the capillaries. The content of the alveoli may be hyaline material or well-preserved red blood corpuscles, which, through rupture of the capillaries penetrate into and fill the alveolar spaces. Careful orientation shows that the so-called alveoli are but cross sections of communicating tubes which possess an intimate relation to the capillaries. The cylindrical cells which line them are derived from the adventitial cells of the capillaries (perithelium). The peritheliomata are therefore tumors which are produced by a proliferation of the capillaries and the cells which separate them from the adjoining lymph spaces, which are

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likewise the endothelium of these lymph channels. For this reason a sharp boundary between perithelioma and endothelioma cannot be drawn.

(*h*) **Cylindroma.**—The various forms of connective-tissue tumors, especially the angiosarcomata and endotheliomata are commonly affected by more or less extensive hyaline transformation or hyaline deposits. In angiosarcoma these deposits are usually about the blood vessels where they form a more or less sharply defined tube of hyaline material. In the *fresh examination* the cellular structure supporting these tubes is lost and the tissue under examination is composed of a structure of glistening homogeneous cylinders. Endotheliomata are occasionally affected by hyaline change when the hyaline material is deposited in the open spaces of the cellular network, in which case the tumor presents an appearance not unlike that of colloid struma (Plate XXXI., Fig. 5), or the cylinders of cells may be transformed into hyaline structures. A similar hyaline degeneration is found, though rarely, in epithelial tumors, in which case it may be impossible to determine the derivation of a tumor.

The forms of sarcoma already described possess varying degrees of malignancy. Some of them, the endotheliomata of the skin, develop very slowly and seldom produce metastasis. In the early stages many of these tumors are encapsulated and the development of the tumor progresses slowly. If, however, the capsule is broken through, the cells of the tumor frequently acquire an increased rate of proliferation and the surrounding tissues are rapidly infiltrated. In other cases, metastases are formed early in the development of the tumor principally through the medium of the circulation. Very commonly the first and only metastatic deposits are in the lungs. The microscopic differentiation of the various forms of sarcoma usually present no especial difficulties. A more difficult task is that of establishing the boundary between certain sarcomata and carcinomata, which possess characteristics in common. (See p. 252.) In many cases it is likewise impossible to distinguish between non-malignant fibroma, especially when the tumor contains a large number of spindle elements, and true malignant sarcoma. The richer the tumor is in cells, the greater the amount of protoplasm in them, the greater the variation in their form and the smaller the amount of intercellular substance, the more serious is the prognosis. When a definite invasion of the surrounding structures, especially the adjacent muscles, can be demonstrated, then the diagnosis of malignancy is established. Further difficulties are occasionally encountered in the differential diagnosis between certain granulation tumors (tuberculosis and syphilis) and true fibrosarcoma and sarcoma. The correctness of Unna's view that the specific granulomata contain a large number of plasma cells while the true sarcomata do not, is far from established. In general the sarcomata are composed of more or less uniform cellular elements, which characteristic should serve to distinguish them from granulation tissue with its various types of cells. No difficulty should be encountered in distinguishing between simple sarcomata and the granulomata of tuberculosis, with their characteristic structure, central necrosis and giant cells. Even in the most atypical tuberculous infections, careful search will most certainly disclose more or less characteristic tubercular structures.

7. Pigmented Nævus and Melanoma.

The nævi pigmentosi are small, flattened or papillary, yellow or brownish thickenings of the skin usually of congenital origin. M. F., F. M. or Beneke's method for the demonstration of protoplasmatic processes, or Mallory's connective-tissue stain.

On examination with the microscope the thickening of the epidermic layer in pigmented nævus is seen to be due to enlargement of the papillæ. These contain columns of cells, arranged perpendicular to the surface of the skin, composed of fairly large epithelial-like elements which are either free from or contain pigment. This pigment is finely granular, resembles closely that of the basal cells of the epidermis in the normal skin and gives a negative reaction for iron. Many observers believe these accumulations of cells are produced by proliferation of endothelium. The nævus cells are usually at some point closely packed about the pigmented basal layers of the papillæ, in which case a sharp differentiation between the pigmented epithelial cells of the skin and the cells of the nævus is impossible. The nævus cells are not all of the same form or size. Between the epithelial-like elements may be found flattened crescentic shaped or branching deeply pigmented forms. The cells of the nævi of young individuals do not possess as much protoplasm as those of older nævi, for which reason the nuclei are more closely arranged. Some forms of pigmented moles do not contain the typical alveoli of nævi cells but are produced by a more or less uniform diffuse enlargement of the papillæ which contain groups of deeply pigmented and stellate cells. These are the so-called chromatophoric cells of the skin. In the older nævi they are usually found in the neighborhood of the groups of nævi cells.

A special form of pigmented malignant tumor which forms widespread metastases, the so-called melanoma, is derived from these pigmented moles and nævi. The primary tumors and the metastases are characterized by their deep brown coloration and spherical form which sharply defines them from the surrounding tissue. When incised the tumor presents a smooth dark brown surface, the scrapings from which, when examined under the microscope, contain large numbers of round and polymorphous pigmented cells. The pigment is in the form of fine and coarser granules and platelets.

M. F., F. M. Carmine.

The melanomata of the skin when examined under the microscope possess an alveolar structure very much like that of nævi, for which reason they are classified as alveolar sarcomata. Occasionally the cells within the alveoli are sharply defined from the stroma and in their arrangement and form are so much like epithelial cells that the tumor presents an appearance more like a pigmented carcinoma than sarcoma. (Plate XXX., Fig. 5.) Careful search of the section will reveal places where the cells of the alveoli appear to be derived from those of the stroma. The distribution of the pigment in these tumors is exceedingly irregular. The cells of the alveoli may contain a small amount of pigment or no pigment at all, while those of the stroma may be heavily laden with pigment. As the stroma of the tumor carries the blood vessels and as hemorrhages in such tumors are common, a certain amount of the pigment may be of the hæmatogenous variety. The occasional detection of iron in such pigment is thus explained. At the periphery of the tumor large branching pigment cells are scattered singly or in groups for some distance in the surrounding tissue. A rather rare form of skin melanoma is composed of pigmented spindle cells and does not possess the alveolar structure of the ordinary melanomata. These tumors usually possess a more or less well-developed fibrillated groundwork which produces an appearance similar to that of the hard fibromata. Melanomata which are derived from the pigmented layers of the choroid are unusually vascular and are classified as pigmented spindle-celled sarcoma.

REFERENCE:

Ribbert. Ziegler's Beiträge, Bd. XXI., 1897.

For an exposition of the facts relating to the epithelial derivation of the cells of skin nævi which has been advocated by Unna for many years see the address of Marchand, Verhandl. d. deutsch. path. Gesellsch. München, 1899.

8. Hæmangioma and Lymphangioma.

It is customary to divide hæmangioma into two forms, the telangiectatic, which is composed of thickened capillaries and the cavernous, in which the tumor is composed of irregularly shaped spaces separated by thin walls. The first-mentioned form occurs most commonly in the skin in the form of flattened, more or less extensive vascular elevations of the surface (nævus vasculosus). To prevent the blood from escaping from the tissue the tumor should be hardened as soon as possible after the operation. M. F., F. M., N. S. Under low power the sweat glands of the skin are surrounded by structures which appear very much like tubular glands cut in various directions. Under high power these are resolved into tubular structures lined with cells which closely resemble cubical epithelium. The presence of blood within the openings, however, indicates that they are altered capillaries. The lining of cubical cells is nothing more or less than the altered endothelium of the capillaries which by the marked contraction of the vessels is elevated and thrown into cubical form. The development of the tumor may be studied at the periphery where the vessels penetrate into the fat tissue. These present an appearance suggestive of a new formation of vessels; at the same time it is extremely probable that the vessels of the tumor are simply the thickened original capillaries of the tissue. Cavernomata occur most commonly in the liver where they form dark red, spongy structures. M. F., F. M., N. S.

Under the microscope the tumor is resolved into a large number of capacious spaces filled with blood. These are separated from each other by a slender connective-tissue structure and the entire tumor is usually separated from the hepatic tissue by a well-defined capsule. Serial sections show that this capsule is continuous with the capsule of Glisson. Included within the tumor are occasional islands of liver parenchyma, as well as isolated liver cells and gall ducts. In other cases the vascular area which composes the tumor is subdivided by slender rows of well-preserved liver cells. In these forms the liver epithelium is in direct apposition to the endothelial lining of the vascular spaces, and shows no evidence of atrophy; on the contrary most of the cells are distinctly hypertrophic. Where the liver is affected by brown atrophy or fatty infiltration, the liver cells within the vascular structure of the cavernoma are unaffected by these changes. The complete isolation from the general circulation of the liver (indicated by the freedom from deposit and infiltration), as well as the occurrence of cavernoma in the livers of new-born children, strongly indicates that they are not produced by dilatation of the ordinary hepatic capillaries, but are of congenital origin. They are probably produced by derangement during the period of development of the proper relations between the epithelial structure and the vascular connective-tissue stroma of the liver. Therefore they should not be classed as tumor formations, but as malformations in the structure of the liver. (Plate XXXII., Fig. 4.)

Circulatory disturbances, thromboses followed by organization and hyaline degeneration of the connective-tissue stroma, have been observed in cavernomata. Tumors which are composed of lymph vessels (lymphangioma) should be classified with the angiomata. Their structure is usually like that of the cavernous hæmangioma, inasmuch as they consist of communicating systems of irregular spaces lined with a simple layer of endothelium. The spaces in these tumors are filled with lymph. As a result of the operation or manipulation

Reference:

The interpretation of liver cavernomata as congenital structures is strengthened by the article of von Schmieden, Virchow's Archiv, 1900.

PLATE XXXI.

Fig. 1.

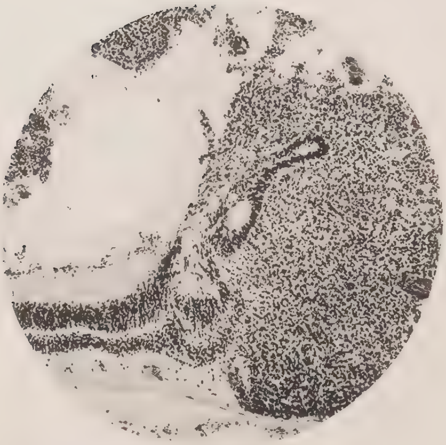


Fig. 2.

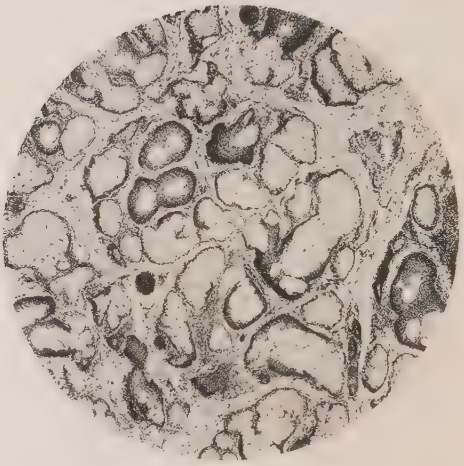


Fig. 3.

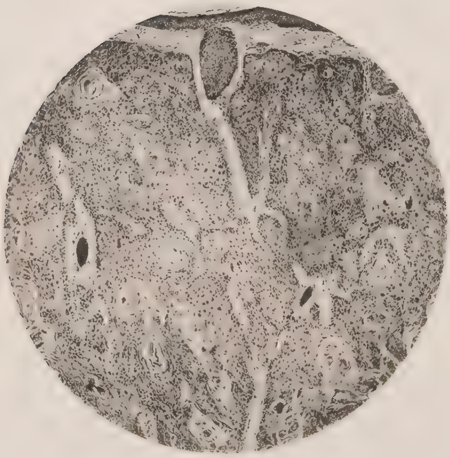


Fig. 4.

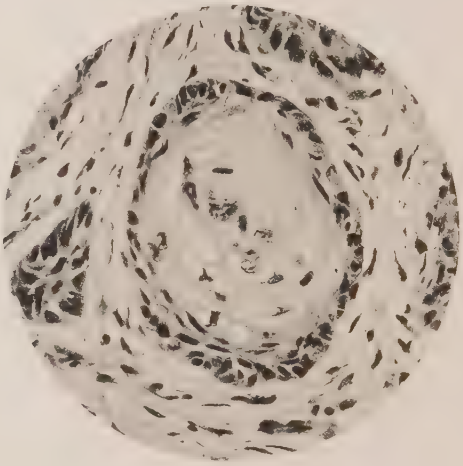


Fig. 5.

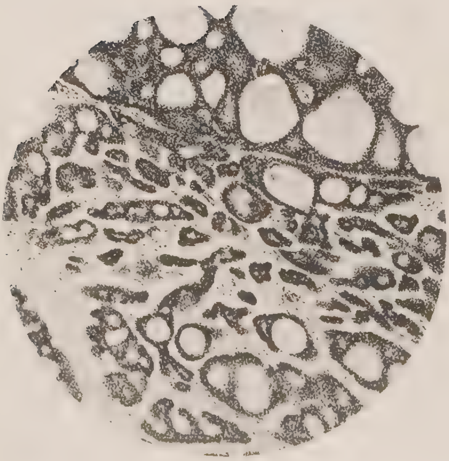
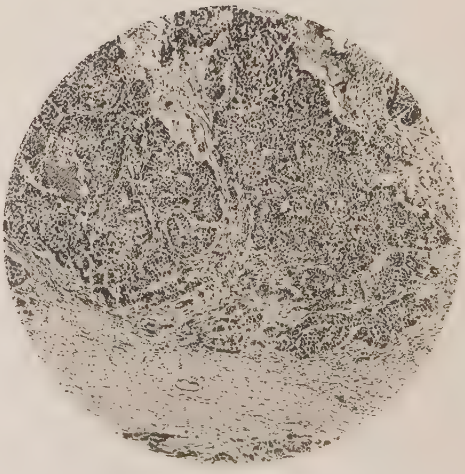


Fig. 6.



[illegible]

before hardening the tumor, a certain amount of blood may become mixed with the lymph and obscure the nature of the growth. If the tumor is directly connected with large lymph vessels these show a well-defined hypertrophy of the muscular structure of their walls.

9. Glioma.

The neuroglia is distinguished from the ordinary connective-tissue structure of the body by the fact that its epithelial derivation can be followed during the later stages of embryonic development or even after birth and that the mature neuroglia can be differentiated from the connective tissue by special staining methods and that its elements possess a characteristic branching form. In describing the pathological processes of the brain and spinal cord the fact was noted that following degenerative processes of the substance of the nervous system the defects were in part repaired by proliferations of the neuroglia (glioses). Besides these localized or diffuse proliferations of neuroglia, a genuine tumor may develop from the neuroglia and produce displacement or invasion of the surrounding structures. A certain number of these tumors are probably of congenital origin as is shown by the occasional discovery within them of cystic spaces lined with ciliated epithelium, which is probably derived by abnormal proliferation or displacement from the epithelium of the median canal.

Material in which the neuroglia is to be stained must be hardened as soon as possible after death. Formaline. F. M. or embed. van Gieson, or further manipulation of the material, Mallory's neuroglia method or Mallory's connective-tissue stain modified for neuroglia. When properly stained the tumor consists of the characteristic spider-shaped neuroglia cells. Beside the processes which are directly connected with the cell, the tumor contains a fibrillated groundwork of detached processes.

Beside the typical neuroglia cells the tumor may include other forms, or be composed of spindle-shaped, multinuclear, branching, or elements closely

REFERENCES:

M. B. Schmidt. Verh. d. deutschen path. Ges. Düsseldorf, 1898.
Stroebe. Ziegler's Beitr., Bd. XVIII., 1895.
Recent literature in Lubarsch-Ostertag, Ergebnisse, V., 1898.

DESCRIPTION OF PLATE XXXI.

FIG. 1. Glioma of the retina. See p. 246. Middle power. Hæmatoxylin. To the left the normal retina with inner and outer granular layers. To the right gliosarcoma composed of round cells. The structure of the tumor is continuous with the inner granular layer of the retina.

FIG. 2. Sebaceous adenoma. Low power. Hæmatoxylin. Glandular structures filled with pale epithelium with small nuclei (spindle cells) (magnifier).

FIG. 3. Squamous epithelioma of the skin (cancroid). See p. 258. Low power. Hæmatoxylin. Above the epidermis. The remainder of the field is composed of proliferating epithelium, in many places formed into concentric structures (epithelial pearls). In many of these evidences of hornification.

FIG. 4. Epithelial pearl. See p. 258. High power. Hæmatoxylin. The concentric arrangement of the flattened epithelium is plainly visible. Many of the cells no longer take the nuclear stain (hornification). About the periphery of the pearl cubical epithelium, similar to the nests of carcinoma cells above and to the left. These cells resemble the basal, cylindrical epithelial cells of the normal epidermis.

FIG. 5. Endothelioma. See page 240. Middle power. Hæmatoxylin. The tumor is composed of cylinders and glandular structures of endothelial cells. Within the openings (above) hyaline material (magnifier).

FIG. 6. Soft carcinoma of the mamma. See p. 256. Low power. Hæmatoxylin. Masses of carcinoma cells invading the connective tissue stroma of the breast. Here and there alveolar arrangement (magnifier).

resembling ganglion cells. In some cases the fibrillated processes are entirely absent or may be discovered only after careful search. So-called gliosarcoma of the retina usually presents this last characteristic. (Plate XXXI., Fig. 1.)

10. Neuroma (Neuro-fibroma).

The term neuroma should only be applied to tumors composed of atypical newly formed ganglion cells or nerve fibers. Such tumors are very rare and when found are usually derived from the sympathetic nervous system.

The so-called amputation neuromata are usually placed in this class. These are nodular thickenings which develop at the amputated ends of nerves and are composed of medullated nerve fibers. The presence of the nerve fibers may be demonstrated by the *fresh examination.* The distribution and arrangement of the fibers can be determined in hardened sections.

M. F., F. M., axis cylinder stain.

Sections of the tumor reveal groups of interspersing, deeply stained axis cylinders held together by a fibrillated connective-tissue stroma. The continuity of the nerve fibers of the tumor with those of the nerve may be demonstrated in sections which include the junction of the nerve end and the tumor. The tumor is the result of regenerative activity at the end of the amputated nerve and the form of the tumor is determined by the resistance of the structures in the stump.

The majority of structures described as neuromata are in fact neuro-fibromata (v. Recklinghausen). These are usually multiple and are distributed in the course of the larger nerves or in the nerve endings, especially those of the skin. They usually appear in the form of soft, flat or polypoid tumors composed of loose connective tissue. The remains of nerve fibers can always be detected in these tumors, and are usually widely separated by connective tissue and show marked atrophic changes.

Tumors of Epithelial Origin.—Epithelial tumors are divided into non-malignant and malignant. The malignant forms are included under the term carcinoma or cancer. The non-malignant remain continuous with the structures from which they are derived. The malignant involve the surrounding structures in the most extensive manner and produce metastases in widely distant organs. In the non-malignant epithelial tumors there is a definite relation between the amount of connective-tissue stroma and epithelium in each case. In the malignant form no relation of this sort exists. The proliferating epithelium is the predominating constituent of the growth. The non-malignant epithelial tumors can be divided into three principal types.

1. The papillary and polypoid epithelial tumors of the skin and mucous membranes.
2. Fibro-adenomatous tumors of glands (*i. e.*, the mamma).
3. Cystic tumors of the ductless glands (*i. e.*, thyroid and ovary).

11. Papillary Fibro-epithelioma of the Skin.

The ordinary warts of the skin are composed of closely arranged projections from the cutis. M. F., F. M., N. S. Picro-carmine. Elastic fiber stain or Gram or Mallory's connective tissue stain.

Under low power the papillary projections which constitute the tumor project far above the surface. They are covered with layers of thickened epithelium

References:

Knauss. Zur Kenntnis der echten Neurome. Virchow's Arch., Bd. 153, 1898.
Beneke. Verhandl. d. deutsch. path. Gesellsch. Düsseldorf, 1898.
Recent literature in Lubarsch-Ostertag, Ergebnisse, V., 1898.

which occasionally extend from one projection to the next and bind them together. The growth is composed of elongated skin papillæ. An excessive proliferation of the epithelium is shown by numerous spherical and oval inclusions, so-called epithelial pearls; onion-like structures of compressed epithelium. The epithelium at the center of these pearls is devoid of nuclear stain (hornified). Where pearl formations develop in the deeper portions of the papilloma they are usually produced by the adherence of the adjacent papillary projections. (Plate XXXII., Figs. 5 and 6.)

In sections stained with picro-carmine these horny pearls are a brilliant yellow and with Gram a deep blue. In the deeper layers of the epithelium occasional isolated nests or projections of epithelium into the surrounding structures may be noted. These are produced by oblique section of the epithelium which dips down between the papillæ. On the surface of the tumor rings of epithelium are occasionally encountered with a connective-tissue center which are produced by cross or oblique section of the papillary projections. The student must acquaint himself with the appearance of these structures and learn to distinguish between artefacts of this nature and the true invasion of the tissue which occurs in carcinoma. In carcinoma the groups of epithelial cells and prolongations which extend into the surrounding tissue are more irregular in form and the cells lack the definite arrangement of the cells of the papillæ, and penetrate into the deep layers of the cutis or the subcutaneous connective tissue. Occasionally the epithelial cells of skin warts are deeply pigmented.

12. Mucous Polyps.

M. F., F. M. or embed. N. S.

Similar papillary and occasionally pedunculated tumors develop from the mucous membranes, especially of the naso-pharynx and large intestine. Microscopic sections which include the constricted base of the tumor show that the peduncle is a prolongation of the submucosa covered by the muscularis mucosa and mucosa. The papillary connective tissue projections upon the surface of the tumor are covered by a layer of epithelium, which is derived from the mucosa. This dips down between the papillary projections where it forms irregularly shaped glandular structures (intestinal polyp). In the large intestine the pseudo-glands thus formed are usually lined with a single layer of epithelium. In rare cases the epithelium of these structures shows variations from the normal. The cells stain more deeply, certain cells are larger than the average, and the pale zone in the superficial portion of the cell body is wanting. The cells are closely packed and the nuclei lie in different planes, suggesting multiple layers of epithelium like those found in the enlarged uterine glands in chronic endometritis. These changes in the epithelium do not indicate malignancy. Before the diagnosis of carcinoma can be made the glands must penetrate through the muscularis mucosa, or the epithelium must show more definite variations from the normal. A transformation of the glands into solid atypical cylindrical structures is almost invariably an evidence of malignancy (adenocarcinoma).

13. Fibro-adenoma of the Mamma.

These tumors appear in the form of well-defined nodular thickenings of the mammary tissues, especially in young persons. When extirpated they may be readily removed from the surrounding tissue and on section the cut surface presents a fibrous appearance. M. F., F. M., N. S. On orientation the connective-tissue structure forms the greater portion of the tumor. The increase in the connective tissue produces widespread separation of the glandular structures of the organ. It surrounds the openings of the lacteal ducts and is especially well developed between the lobules of the mammary glands. The newly formed tissue may be distinguished from the mature interacinous connective tissue of the organ by the number of nuclei which it contains and the intermingling of the fibrous bands. When the connective-tissue structure is especially well developed about the openings of the mammary glands the tumor is known as a pericanalicular adeno-fibroma. The epithelial structure within the tumor likewise shows an increase over that of the normal organ. This increase in the glandular structure may affect the acini of the glands, or more particularly the smaller lacteal ducts are greatly increased in size and number. The latter usually produce closely packed stellate or branching figures (tubular form). (Plate XXXII., Fig. 3.)

In some fibro-adenomata of the mamma the structure of the original glands may no longer be recognized. The tumor consists of an irregular system of glandular canals supported by a uniform connective-tissue structure. These canals possess the structure of the normal milk glands, that is, they are lined with a single layer of cubical epithelium which rests upon a supporting structure of slender spindle connective-tissue cells, which directly beneath the epithelium is condensed into a well-defined membrana propria. The enlarged lacteal ducts occasionally become occluded, which is followed by cystic dilatation of complete segments of the tumor (cystic fibro-adenoma of the breast).

A third form of non-malignant mammary tumor is the so-called intracanalicular fibro-adenoma. The enlarged lacteal ducts and glands of these tumors are surrounded by a well-developed connective-tissue stroma, which, however, projects into the lumenæ of the canals in the form of polypoid structures and reduces them to mere irregular openings and clefts lined with epithelium. Closure of the canals may likewise produce cystic dilatation which reveals more clearly the polypoid projections of connective tissue. Cross sections of the polypoid projections produce isolated islands of connective tissue surrounded by a single layer of epithelium within the dilated canals or cysts. Beside the fibro-adenomata simple adenomata of the breast develop which are not associated with increase of the connective-tissue stroma. These consist of proliferation of groups of acini and are usually accompanied by more or less excessive dilatation of the ducts. The mamma is more dense than normal, and the proliferated acini protrude above the cut surface of the organ, giving it a coarse granulated appearance. Beside the elevations of the surface small cysts filled with brownish or yellowish material may be noted (so-called chronic cystic mastitis). M. F., F. M., N. S.

Under the microscope the enlarged acini are grouped together and the proliferation is confined to single lobules. Scattered between these are perfectly normal glands. About the periphery of the enlarged lobules the lacteal ducts are enlarged or dilated into small cysts. Within these the epithelial lining presents an unusual status. Single epithelial cells are swollen and contain large pale nuclei. In many places the epithelium forms multiple layers and the boundary of the lumen is less distinct than normal. The irregularity of the openings of the canals and cysts is the result of bud-like projections of groups of cells or single cells into the lumen. Or a complete network of cells may obscure the openings of the canal. The tunica propria may be plainly discernible.

These epithelial proliferations should always be regarded with suspicion and where present a careful search of the preparation should be made for isolated nests of epithelium. When present these are embedded in the connective-tissue stroma and show no trace of a tunica propria; the cells in them possess the characteristics of the enlarged pale epithelium of the canals, their isolation

PLATE XXXII.

Fig. 1.

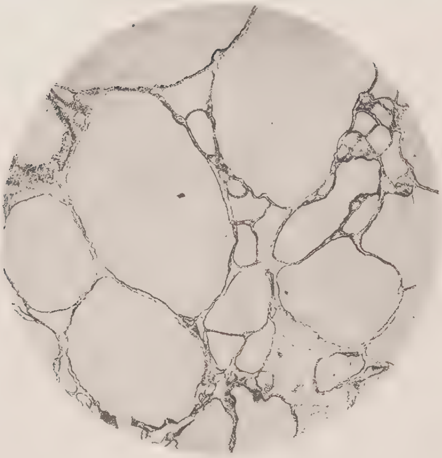


Fig. 2.

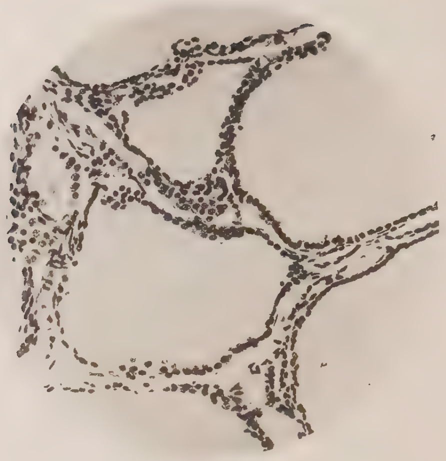


Fig. 3.

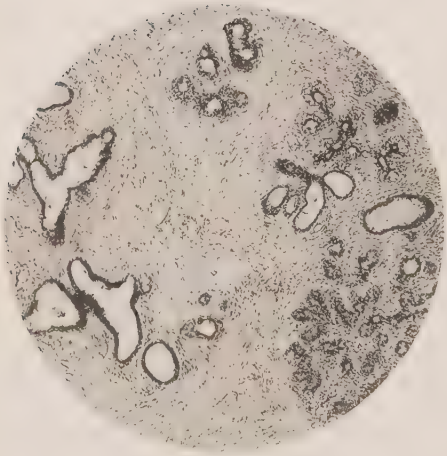


Fig. 4.

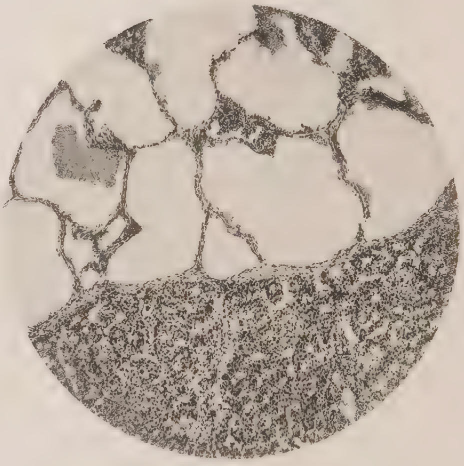


Fig. 5.

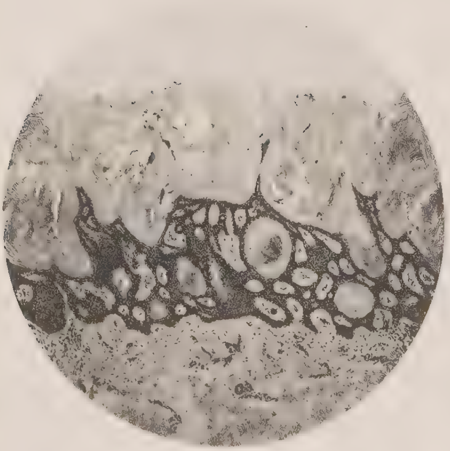
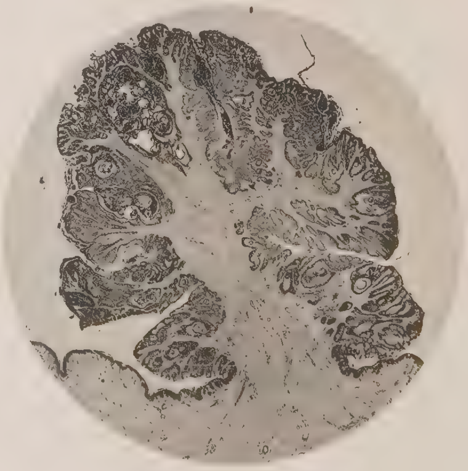


Fig. 6.



marks the beginning of a malignant change (mastitis chronica carcinomatosa). Round-celled infiltration of the connective tissue may or may not accompany the changes in the epithelium.

14. Struma.

Enlargements of the thyroid whether diffuse or nodular are included under the heading struma. These enlargements may be due to increase in the epithelial constituents or the connective-tissue structure of the organ.

(*a*) **Struma Parenchymatosa Nodosa.**—The cut surface of the enlarged thyroid shows nodular elevations and the coarse granulated structure of the normal gland. M. F., F. M., N. S., or harden by cooking, embed, picro-carmine or van Gieson.

The nodular projections of the cut surface of the organ may be recognized in sections as closely packed alveolar or tubular structures which are lined with the characteristic epithelium of the normal alveoli of the thyroid. The nodular enlargement is therefore due to dilatation and proliferation of the specific thyroid structures. The derivation of these enlarged tubular alveolar structures from the normal parenchyma of the organ is shown by the colloid material which they contain. When the increase of colloid material affects not only the newly formed but the remaining structure of the thyroid the organ may become greatly enlarged, producing the condition known as colloid struma. (Plate XXXII., Figs. 1 and 2.)

Under low power the marked dilatation of the alveoli fixes the attention. They are filled with a homogeneous material which in hæmatoxylin preparations remains unstained but in preparations hardened by cooking and stained with picro-carmine is a bright yellow, or in van Gieson preparations a brownish color. Under high power the typical cubical structure of the epithelium lining

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DESCRIPTION OF PLATE XXXII.

Fig. 1. Colloid struma. See p. 249. Low power. Hæmatoxylin. Large, irregular openings lined with a single layer of epithelium. The cavities are filled with a homogeneous colloid material.

Fig. 2. Colloid struma. See p. 249. High power. Hæmatoxylin. Note the characteristic appearance of the epithelium (thyroid epithelium). The colloid material in the small alveolus above shows marked retraction from the epithelium lining the space.

Fig. 3. Fibro-adenoma of the breast. See p. 248. Low power. Hæmatoxylin. To the right and below a group of lacteal glands. To the left above and below larger and smaller lacteal ducts in transverse and oblique section. These are separated and surrounded by a dense fibrous connective-tissue structure.

Fig. 4. Cavernoma of the liver. See p. 244. Low power. Hæmatoxylin. Below normal parenchyma of the liver, which shows no evidence of compression. Above and separated from it by a well-defined capsule, a series of irregular cavities from which the blood has been removed during the process of hardening. One small opening above the center filled with blood corpuscles. Cavities separated by thin walls.

Fig. 5. Flat fibro-epithelioma of the skin. See p. 247. Low power. Hæmatoxylin. Below the subcutaneous connective tissue. Upon this the cutis in somewhat oblique section. The papillary bodies owing to the oblique section are scattered through the deeply stained epithelial structure and appear as pale openings containing nuclei. Between these well-defined epithelial pearls with concentric striation. Above a thick layer of hornified epidermis (magnifier).

Fig. 6. Polypoid fibro-epithelioma of the skin. See p. 247. Very low power. Hæmatoxylin. To the left and right normal epithelium of the skin. Central projection derived from the subcutaneous connective tissue. Upon this cauliflower-like proliferation of the epithelial coverings of the skin. Papillary projections here and there adherent and including between them large epithelial pearls.

the alveoli may be seen. The colloid material does not come in direct contact with the epithelium but is separated from it by a well-defined space. Fine projections of the colloid material extend between the epithelial cells and attach it to the walls of the alveoli. It is still a matter of discussion whether this lack of apposition between the colloid material and the epithelium is due to retraction of the colloid mass or the presence of a clear secretion between the colloid and the epithelium. The fact that separation, excavation and vacuole formation of the colloid material may be detected in fresh preparations speaks for the latter possibility. In large goiters homogeneous masses of material closely resembling colloid are found in the connective-tissue stroma. It is possible that this material is colloid substance which has found its way into the lymph channels.

(*b*) **Struma Fibrosa.**—Although this form of enlargement of the thyroid does not produce an epithelial tumor it is well to consider it in connection with the parenchymatous form of struma. The change usually occurs in the form of dense or partially calcified nodules in the thyroid. M. F. (when necessary decalcify), F. M., N. S., van Gieson or Mallory's connective-tissue stain.

In microscopic section the connective-tissue stroma overbalances the glandular structures. This predominance of the connective tissue may be so extensive that even in large sections only a few compressed and atrophic alveolar structures remain. The newly formed connective tissue may become fibrous, may be affected by hyaline change, contain more or less extensive deposits of chalk or an unusual number of blood vessels (struma fibrosa, calcificans, vasculosa). The remains of previous hemorrhages in the stroma may be found in more or less extensive deposits of hæmatogenous pigment.

15. Cystic Tumors of the Ovary.

The form of tumor most commonly encountered in the ovary are the cystomat a Two varieties:

(*a*) **Glandular Cystoma.**—The glandular tumors of the ovary are usually formed by larger and smaller cavities lined with epithelium (multilocular cystoma). The content of the cavities in some cases is clear, in others cloudy, almost purulent in appearance and may be either thin or thick and tenacious, like mucus. The microscopic examination of the *fresh fluid* from the tumor discloses the presence, when the fluid is clear, of a certain number of cylindrical epithelial cells, usually placed side by side. When the fluid is cloudy and resembles pus it contains a large number of true pus cells, recognized by their horseshoe nuclei. These show a high grade of fatty degeneration. In the tenacious mucus-like material are either goblet cells or cells affected by vacuolous dilatation. These apparently contain mucoid material. Treatment with acetic acid fails to produce the typical thready coagulation which characterizes mucin, showing that the material merely resembles mucus.

M. F., F. M. or embed. N. S., F. A. Embed, special mucus stain or van Gieson.

The microscopic investigation of a section made through the wall of one of the larger cysts reveals (low power) gland-like structures which penetrate into the connective tissue which surrounds the cyst wall. Many of these show the beginning of cystic dilatation and reveal the method by which the larger cystic structures are formed. Under high power the epithelial lining of the cysts appears more or less regular and is continuous with the lining of the glandular projections about the periphery. The epithelium is of the high cylindrical type with basal nuclei. The protoplasm of the cell-body near the free surface is pale. Here and there between the high cylindrical epithelial elements will be found groups of typical goblet cells.

(*b*) **Papillary Cystoma.**—Papillary cystomata usually include but one or only a limited number of cavities. These cavities are filled in by papillary elevations of the cyst wall. Many papillary cystomata are derived from the parovarium. The content of the cysts is usually thinner than that of the glandular cystomata. Scrapings from the surface of the tumor examined in salt solution disclose the presence, especially in fresh tumors, of well-preserved ciliated epithelial cells. The cilia of these cells, especially if the tumor has been kept warm, may be in active motion.

M. F., F. M. or embed. N. S.

Microscopic sections of the tumor disclose the nature of the papillary elevations of the cyst wall. These possess connective-tissue axes, which sends out projections in all directions. (Plate XXXIV., Fig. 3.) The papillary projections, as well as the smooth portions of the cyst wall are covered by a single layer of high cylindrical epithelium upon the surface of which the cilia when intact may be recognized. In the hardened section an occasional goblet cell may be detected between the cylindrical epithelial elements. In rare cases the connective tissue stroma may contain spherical chalk concretions like those found in psammoma. Although the papillary connective-tissue structures usually project into the cystic center, they sometimes develop upon the surface of the tumor.

A sharp division between the glandular and papillary form of cystoma cannot be made. On the contrary papillary projections are very common in the walls of the glandular form and isolated glandular structures appear in the papillary form. The differentiation of these tumors from carcinomata is sometimes attended with difficulty. The greater the predominance of the glandular structures, the greater becomes the possibility that the tumor is of a malignant type. A diagnosis of malignancy cannot be made with safety unless metastases are formed. In the papillated form of cystoma where the papillæ are developed upon the surface, these may be implanted upon the peritoneal surface, and develop into numberless new tumors. This phenomenon is usually considered a form of transplantation through contact and is not a form of true invasion by proliferation.

Both forms of cystoma may undergo carcinomatous transformation. This change may be suspected when the epithelium lining the glandular cavities or covering the papillary projections shows certain deviations from the normal, which consist of obliteration of the distinguishing features of the mucous and ciliated epithelial elements with the development of an indifferent type of cylindrical epithelium. The altered epithelium possesses marked proliferative energy and forms irregular multiple layers so closely arranged that distortion of the cells and nuclei is frequent. In places where the proliferation is excessive elevations in the epithelial mass are produced. Following these changes solid irregularly formed cylindrical projections of the epithelium invade the connective-tissue stroma. When these changes are present no doubt of the malignant character of the tumor may be entertained.

16. Carcinoma (Cancer).

Infiltrating epithelioma or cancer produces tumors which are not limited by the boundaries of the structures from which they are derived. They not only

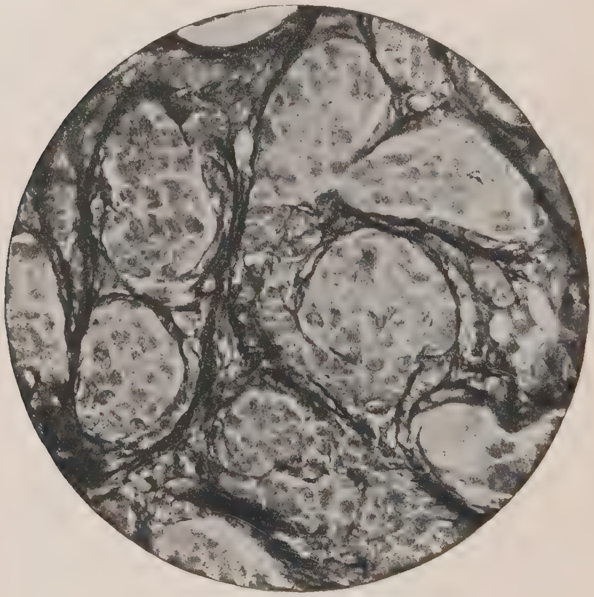
REFERENCE :

For the literature of cystoma of the ovary see *v. Kahlden*, Ziegler's Beitr., Bd. 27, 1900.

displace but invade and destroy the adjacent tissues. The excessive proliferative energy which they develop is shown by numerous metastases which in contradistinction to sarcoma are usually produced through the medium of the lymph channels. As the primary growth may be very small and for this reason the microscopic diagnosis in doubt, it is always necessary to investigate the adjoining lymph nodes, as these occasionally show more well-defined changes than the affected organ.

Like all other tumors, cancers possess a vascular connective-tissue stroma which supports the epithelial elements of the growth. (Fig. 37.) The greater the amount of stroma the denser the tumor, and vice versa, where the epithelial elements form the greater part of the growth, the softer the structure. Scirrhus or hard carcinoma can be differentiated from fibro-sarcoma by the denser character of the stroma, and the presence of epithelial nests which may occasionally be recognized with the naked eye. In more difficult cases the macroscopic differentiation fails. In the softer forms the epithelial character of the tumor may be more easily recognized. If a fresh tumor of this variety be compressed, a thick fluid is squeezed out and the epithelial contents of the nests are extruded in the form of yellowish white plugs and spiral or vermiform structures upon the cut surface. If this material be examined in the *fresh state* it is composed of cylindrical or more cubical or large, irregularly shaped epithelial cells usually in an advanced state of fatty degeneration. They possess large vesicular nuclei, which are plainly visible when the preparation is treated with acetic acid. The fibrillated intercellular substance so characteristic of sarcoma is entirely wanting. This difference is, however, not fundamental as in alveolar sarcoma an analogous arrangement of the stroma and the cells of the tumor may be found. The form of the cells does not in itself suffice for the purpose of differential diagnosis for in many carcinomata, spindle-shaped elements may be found which closely resemble those of certain sarcomata and in many peritheliomata the cylindrical epithelium closely resembles that found in the ordinary forms of adeno-carcinoma. Likewise the form of the nuclei is not characteristic in either of these forms of tumor and all that has been stated regarding the variability of the nuclei in sarcoma, applies as well to carcinoma. The cells of the soft variety of cancer are less sharply defined from the stroma which in most cases is poorly developed. From the cut surface of these tumors a large amount of fluid may be expressed, which, according to the origin of the tumor, is composed of epithelial cells with more or less well defined characteristics. In many of these cases the

Fig. 37.



Soft carcinoma of breast showing stroma. Mallory's connective tissue stain. (High dry power.)

differential diagnosis between soft sarcoma and carcinoma becomes difficult. The location and extent of the tumor and character of the metastases must all be considered before a diagnosis can be made. As we have already noted the fresh examination is of especial interest in carcinoma, in order that we may determine the extent of the invasion and whether or not the operation has included the entire growth. In beginning the investigation of the tumor this should be borne in mind and the cut surfaces made by the surgeon should be carefully scanned for the presence of larger or smaller deposits and when suspicious structures are found, these should be carefully examined under the microscope. Like all of the malignant tumors, the larger or smaller nodules of cancer are distinguished by their whitish appearance and hardness. For this reason the smallest carcinomatous axillary lymph nodes may be readily palpated. In larger lymph nodes the carcinomatous infiltration may be recognized on incision by white lines or dots in the grayish-red substance of the node. Investigations of hardened sections of carcinoma reveal in typical cases the sharp differentiation between the connective-tissue stroma and the carcinomatous cells. These are usually arranged in spherical or oval groups, embedded in the connective tissue, forming the so-called alveoli. The cells within the nests are closely packed together usually without trace of intercellular substance. These two characteristics, the alveolar arrangement of the cells with sharp differentiation from the stroma and the absence of fibrillated intercellular substance, distinguishes infiltrating epithelioma. A certain amount of shrinkage of the alveolar nests follows the action of most hardening agents and they are thus separated from the surrounding connective tissue by a narrow cleft which more sharply accentuates the differentiation between the cells and the stroma. This characteristic might serve to distinguish carcinoma, had we not already noted that in the alveolar forms of sarcoma, the sarcoma cells are at times more or less sharply defined from the stroma, and the fibrillary intercellular structure is absent. In typical forms of carcinoma the cancer cells closely resemble those of the epithelial structure from which the tumor is derived. Thus a form of epithelioma is encountered in the intestinal tract where the alveoli are lined with high cylindrical epithelium, so arranged as to closely resemble the glandular structures of the mucous membrane (so-called adeno-carcinoma). The pseudo glands of this tumor are, however, not provided with a tunica propria and possess no excretory ducts. In some forms of carcinoma the alveoli are lined with flattened, horny epithelium. In these cases the epithelial nature of the tumor is easily determined, although in certain rare forms of endothelioma and perithelioma, the cells are so arranged as to closely resemble them. The greatest attention should always be given to the investigation of the periphery of the tumor. In malignant tumors the boundary is always marked by a more or less well defined round-celled infiltration of the adjacent structures, probably representing a reaction of the tissue against the invasion of the carcinoma. In non-malignant tumors the round-celled infiltration is absent, unless the tumor has been irritated or is ulcerating.

The various forms of carcinoma may be properly classified as:

1. The glandular carcinomata (adeno-carcinoma, malignant adenoma).
2. Solid, soft and hard cancer (carcinoma solidum).
3. Squamous epithelioma (cancroid).

(*a*) **Glandular Carcinoma.**—Carcinomata of the gastro-intestinal tract are usually of the glandular type. Microscopic sections (M. F., F. M., N. S.) of the tumor should be so laid as to include the point of transition of normal mucous membrane into carcinoma. When examined beyond the point of transition a sharp differentiation of the layers of the intestinal wall and the mucous membrane with its crypts of Lieberkühn lined with goblet cells and cylindrical epithelium, the nuclei of which, arranged near the base and the outer zone of pale protoplasm, may be readily recognized. In the region of transition the submucosa is thickened, elevating the mucosa which ends abruptly, at the point where the carcinoma begins. (Plate XXXIII., Fig. 3.) Before this occurs the mucosa shows various changes. In the first place a well-developed small-celled infiltration between the glands. The greatly thickened submucosa is permeated with round cells and contains a large number of new blood vessels. At the same time these structures are sharply divided by the muscularis mucosa. At the point of actual transition there suddenly appears between the normal glands of the mucous membrane, irregularly formed, more or less continuous glandular structures, the epithelium of which shows marked variation from the normal. These cells are arranged in multiple layers, are much more closely packed and are of an entirely different nature. (Plate XXXIII., Fig. 4.) The protoplasm is much darker and more finely granular than in the normal intestinal epithelium. The nuclei are much richer in chromatin and show great variation of form and size. In general these cells are cylindrical in form, although the close arrangement produces certain modifications. In spite of the great variety of form in the cells a more or less typical glandular structure is preserved, in which may be recognized a lumen, lined with multiple layers of epithelium but without a tunica propria. In the region of transition these structures are scattered through the mucosa into which they penetrate and represent the farthermost projections of the carcinoma. In the region of the tumor proper an entirely new characteristic develops. This is found in the destruction of all of the boundaries of the mucous membrane including the muscularis mucosa by the extensive atypical glandular proliferations of the tumor. These extend beyond every physiological boundary and penetrate even into the muscularis.

The obliteration of the structures of the intestinal wall is assisted by a more or less well-defined round-celled infiltration which in combination with the carcinoma obscures all landmarks. The presence of the atypical glandular structures, but especially the changes in the epithelium insure the diagnosis of carcinoma. In other portions of the tumor the changes in the epithelium are not associated with the same evidence of proliferation noted in the tumor proper. In these regions, instead of the peripheral extension of the atypical glands a transformation from normal into carcinomatous epithelium occurs in the glandular structures of the mucosa. The first change noticeable is the absence of the goblet cells. The cylindrical epithelium loses its characteristic form and becomes more slender, the nuclei appear in the center, or when the cells are more closely packed occupy different elevations, producing an appearance like multiple layers of epithelium. Here and there more active proliferation produces bud-like projections of cells which give the lumen irregular outlines. Where these elevations of the surface are sufficiently extensive the lumen may be markedly encroached upon or totally obliterated. Probably as a result of pressure from the extensive proliferation the tunica propria rapidly disappears. During this stage of carcinomatous change numerous karyokinetic figures appear in the epithelium. These, in contradistinction to the normal karyokinetic figures, in which the nuclear spindles are parallel to the axes of the glands, show various modifications, not only in the direction which the plane of division bears to the axis of the gland, but in the form and structure of the figures themselves. As we approach the tumor we find that the carcinomatous glands extend deeper and deeper into the coats of the intestinal wall, until they penetrate into the muscularis and are lost in the irregular structures of the tumor proper. The transformation of normal into carcinomatous epithelium may be sudden, so that unaltered and carcinomatous glands lie side by side, or even a few cells in a gland may show the carcinomatous change and be arranged in immediate apposition to normal epithelium. In viewing such structures it is well to remember that carcinomatous glands may penetrate from the submucosa between the unaltered glands of the mucosa. Where this occurs one must guard against an improper interpretation of the relations which exist, as the process is usually one of secondary adhesion of unaltered mucosa to the proliferating carcinoma (Ribbert). On the other hand the examination of beginning carcinoma leaves no doubt that primary carcinomatous degeneration of the glands and primary penetration into the structures of the intestinal wall do occur. It is exceedingly interesting to note that in these cases the carcinomatous transformation is not necessarily associated with carcinomatous proliferation; that is, the penetration of the structures into the muscularis. Likewise glandular stuctures will be found which penetrate through the tunica propria into surrounding structures, and beside their increase in length, show no carcinomatous changes in the epithelium. For this reason we must conclude that the morphological changes in the epithelium are only outward expressions of a biological transformation. This outward expression usually accompanies the carcinomatous change but is not of necessity associated with it. On the other hand, it must be remembered that similar changes in the epithelium, the deep staining of the protoplasm, the disappearance of the goblet cells and the multiple layer formation are found in simple polypoid growths in the large intestine, and metaplastic transformation of the specific glandular epithelium of the stomach into simple cylindrical epithelium is a common occurrence in the atypical proliferations which surround the margins of contracted ulcers. In themselves, these changes do not warrant a diagnosis of cancer. Where they are observed the process must be considered of a suspicious character, but the final diagnosis may only be made when the proliferating glands extend through the muscularis mucosa and invade the surrounding structures.

(*b*) **Mucoid Carcinoma.**—This form of cancer is commonly encountered in the gastro-intestinal tract, especially the stomach. It may be recognized macroscopically by the mucoid semi-transparent character of the cut surface. The penetration of the carcinoma between the bands of muscle may be detected with the naked eye as semi-translucent bands and drops. M. F., F. M., N. S. or methylene-blue. Under low power the muscular structures are here and there widely separated by unstained cavities sometimes lined with a continuous layer of epithelium. This may be wanting when the clear space usually contains isolated epithelial elements embedded in the colorless mass which fills the cavity. (Plate XXXIII., Figs. 2 and 5.) Here and there the cells possess a more well-defined glandular structure or are arranged in compact cylinders which penetrate into the connective-tissue stroma or between the muscle fibers. Between these and the above-described cavities in the tissue transition structures may be noted. Under high power the solid epithelial structures are composed of cylindrical or polymorphous cells usually closely packed. The appearance of these cells is variable, the cylindrical forms simulating the goblet cells of the gastric mucosa. The polymorphous forms possess a finely granular or more transparent, or a widely distended cell body. In the latter case the form of the cell is spherical and the nucleus is pressed against the outer margin of the cell by the clear contents. This form of cell is most frequently found in the clear

References:

Hauser. Das cylinderepithel Carcinom des Magens und des Dickdarms. Jena, Fisher, 1890.

Kaufmann. Unters. ueber das sog. Adenoma malignum speziell desjenige der Cervix uteri, etc., Virchow's Archiv, Bd. 154, 1898. Likewise Ribbert, p. 304.

spaces above described. The intercellular material is thready or finely granular and in ordinary hæmatoxylin specimens is unstained. Beside the well-defined cells the remains of protoplasm or cell nuclei are embedded in this material. (Plate XXXIII., Fig. 5.) This stringy, granular material closely resembles mucus and owes its granular and stringy appearance to the action of the fixing agent. A close resemblance of this material to mucus is found in its reaction to specific staining methods (harden in M. F., stain with methylene-blue). When stained with methylene-blue the clear spaces of the tissue take on an intense blue color. (Plate XXXIII., Fig. 1.) Within the cells the beginning mucoid change takes the form of deeply stained (blue) portions of protoplasm. This appearance of the mucoid material within the cells indicates that it is a secretion product, which is liberated when the cell disintegrates.

(*c*) **Solid, Soft Carcinoma.**—In contradistinction to glandular carcinoma, which is recognized by its characteristic structure, and mucoid carcinoma which is distinguished by the special product of its activity, the mucoid material, the ordinary soft carcinomata exhibit no special function or characteristic structure. They most frequently develop in the glandular organs, especially in the breast. M. F., F. M., N. S. By low power the tumor may be recognized as masses of closely packed epithelial cells. These bear no definite relation to each other, and exhibit great variety of form. A common appearance is a more or less well defined net-like arrangement of the cells, which is produced by the distribution of the cells through the lymph vessels or the lymph spaces of the organ. Nodular formations in this network are likewise due to the characteristic structure of the lymph vessels. The malignant character of the tumor is well seen at points where the cells penetrate into the mammary fat. (Plate XXXIV., Fig. 1.) For this reason sections should be made which include the periphery of the tumor and the adjacent fat tissue. A more or less well defined round-

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DESCRIPTION OF PLATE XXXIII.

FIG. 1. Mucoid carcinoma of the stomach. See p. 256. Low power. Methylene-blue. The mucoid material is stained a deep blue obscuring the alveolar structure of the carcinoma. Running through the center of the field, section of the pale connective-tissue stroma.

FIG. 2. Mucoid carcinoma of the stomach. See p. 256. Middle power. Alum carmine. Mucoid material unstained. Below, a bundle of smooth muscle fibers. Above, a similar bundle. Between these unstained mucoid material containing more or less degenerated carcinoma cells. The remains of an alveolar structure may be distinguished.

FIG. 3. Malignant adenoma of the rectum. See p. 254. Very low power. To the left a section of the unaltered large intestine. At the center a sudden projection upward of the submucosa. The left of this projection is still covered by the unaltered mucous membrane of the intestine. Beyond this first projection and to the right large irregular glandular structures extending into the thickened submucosa. The normal mucous membrane ends sharply in a cleft above. A direct transformation of the epithelium of the mucous membrane into carcinomatous epithelium cannot be traced in this section. The carcinomatous nature of the glandular structure to the right is shown by the invasion of the submucosa, the muscularis mucosa having been broken through.

FIG. 4. Malignant adenoma of the rectum. See p. 254. High power. Hæmatoxylin. Irregular glandular structures in the submucosa. A membrana propria is wanting and the epithelium consists of irregular multiple layers. Nuclei deeply stained, no goblet cells visible (magnifier).

FIG. 5. Mucoid degeneration of carcinoma cells. Carcinoma of the cæcum. See p. 255. High power. Alum hæmatoxylin. The degenerating cells are embedded in mucoid material. To the right somewhat above a carcinoma cell with clear space in the protoplasm, the nucleus pushed to one side (beginning mucoid degeneration). Scattered between the carcinoma cells a number of leucocytes.

PLATE XXXIII.

Fig. 1.

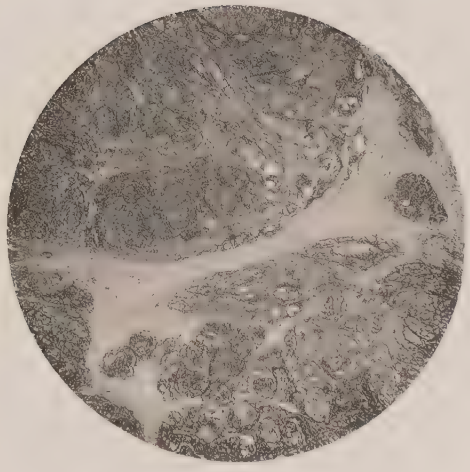


Fig. 2.

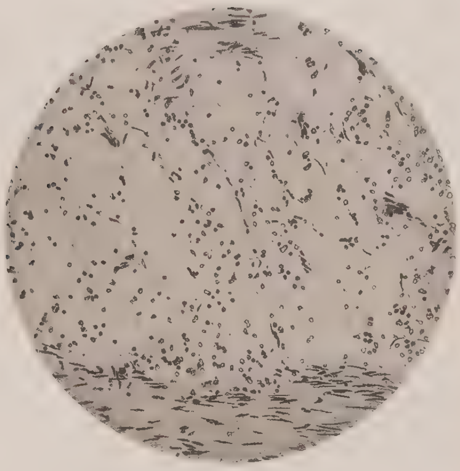


Fig. 3.

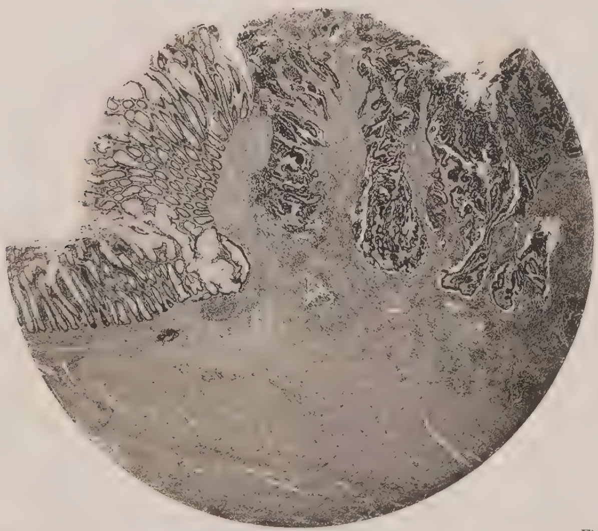


Fig. 4.

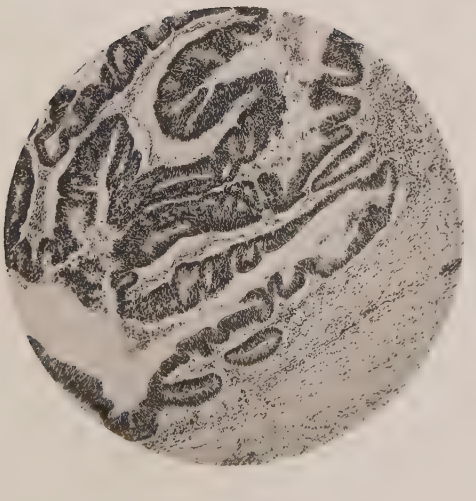
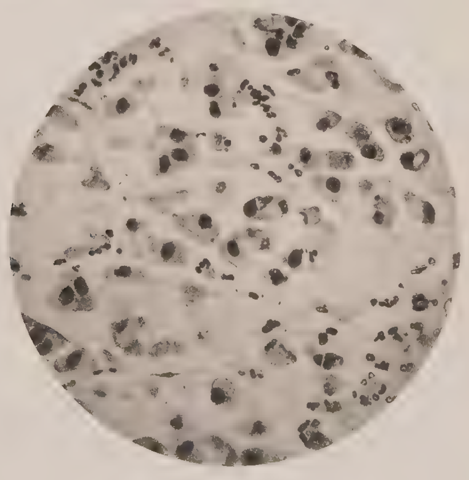


Fig. 5.



celled infiltration is likewise found in this region indicating the usual reaction of the invaded structures. The character of the invading cancer cells may be well observed at the margin of the growth. They follow no regular arrangement, and are not bounded by a membrana propria. Here and there the carcinomatous cells penetrate into the walls of the blood vessels which should be carefully searched (elastic fiber stain).

(*d*) **Hard Carcinoma (Scirrhus).**—This form of cancer is distinguished from the soft form by the predominance of the connective-tissue stroma over the carcinoma cells. They are most frequently found in the female mamma where they produce a characteristic retraction of the surface or the nipple. M. F., F. M., N. S. In contradistinction to hard fibro-adenoma, the scirrhous cancer is firmly fixed to the surrounding structures and cannot be removed without excision of more or less extensive portions of the organ. Under the microscope the connective-tissue stroma is greatly in excess of the small alveoli filled with epithelial cells. (Plate XXXIV., Fig. 2.) These are usually small, poor in protoplasm and appear as if compressed by the surrounding connective tissue. The stroma is frequently affected by hyaline metamorphosis. About the periphery of the tumor fresh carcinomatous proliferations are found, which are identical with those described as occurring about the periphery of the softer forms of carcinoma. In all cases of carcinoma of the mamma, it must be especially borne in mind that even the smallest tumors may lead to early metastases in the axillary lymph nodes, for which reason they should invariably be removed with the primary tumor. Even when the axillary nodes show no considerable swelling or other changes, beginning metastatic deposits may be found by the microscopic examination.

(*e*) **Squamous Epithelioma (Cancroid).**—The characteristic form of carcinoma which develops from the structures of the skin is the so-called squamour epithelioma or cancroid. They are most frequently found in the region of ths lip, about the borders of chronic skin ulcers or in the neighborhood of lupue patches. The tumor may be recognized by its unusual hardness and the mannes in which it invades the subcutis or even the underlying muscular structures. The fresh *preparation reveals* the specific elements of the growth. These are either large round spindle-shaped or crescentic epithelial cells. By reducing the illumination it is often possible to observe fine protoplasmatic projections at the edges of the isolated elements. When viewed from above these protoplasmatic projections resemble fine points arranged in more or less regular rows. They represent the persistence of the fine protoplasmatic projections of the normal cells of the epidermis, the so-called prickle cells. Besides the isolated elements many of the cells are arranged in concentric fashion in the form of isolated spherical bodies, the so-called epithelial pearls. Where the epithelial pearls are broken the laminated appearance of the structure becomes more apparent. M. F., F. M., N. S. or picro-carmine, or Gram or van Gieson.

In describing the lesions of lupus we noted that pathological proliferation of the papillæ commonly resulted from the irritation of the toxic substances elaborated by the tubercle bacilli. Likewise in papilloma of the skin the tumor is in part formed by epithelial proliferation. In neither of these cases does the growth of epithelium extend beyond the boundaries of the cutis. The distinguishing feature of squamous epithelioma is the disregard which the proliferating epithelium shows for the normal boundary of the cutis. The determination of this fact is rendered somewhat difficult by a marked increase in the tissue of the subcutis which causes more or less extensive elevation of the cuticular layers. For this reason the apparent failure of the tumor to penetrate into the underlying structures does not determine whether the limits of the cutis have been passed. Therefore we must carefully observe the relation of the proliferating epithelial structures to the sweat glands, the hair follicles and the larger vessels. When the cells of the tumor surround or come in close contact with these structures they have passed beyond the physiological boundary and the proliferation is of a carcinomatous nature. (Plate XXXI., Fig. 3.) Like all other forms of carcinoma, the periphery of the new growth is bounded by an extensive round-celled infiltration. Occasionally the elements of the tumor invade the lymph spaces when a more or less complete network of carcinoma cells is found. The projections of the tumor show a characteristic laminated appearance very much like the normal layers of the skin. The outer portion of the projection is separated from the connective tissue by a basal layer of cylindrical epithelium. The central portion is composed of prickle cells which in the center of the structure are more or less flattened. Here and there they are formed into spherical laminated structures, the epithelial pearls. (Plate XXXI., Fig. 4.) The cells of the outer layer of the pearls possess nuclei. Those near the center show complete hornification and when stained with picro-carmine take on a brilliant yellow color, or with Gram a deep blue, in case the tissue has been hardened in alcohol. In many cases of squamous epithelioma the characteristic structure of the cells is not well defined. Variations in the form and arrangement of the nuclei, as well as changes in the cells, similar to those described in sarcoma, may be encountered.

The epithelial layers of the skin and the structure of the tumor is infiltrated with leucocytes and migratory cells which some consider to be parasitic amœbæ (Isen). These penetrate between the cells and are commonly found within the epithelial pearls, either lying between the laminated layers or forming a group at the center.

(*f*) **Metastatic Deposits of Cancer.**—We have already noted in describing the pathological changes of the lymph nodes, that the transportation of carcinoma cells is usually by way of the lymph spaces. Where the primary cancer is in one of the large abdominal organs the metastases are usually formed through the medium of the blood vessels. For this reason gastric and intestinal carcinoma are commonly followed by secondary growths in the liver. These metastatic deposits may take one of two forms:

1. **Nodular Secondary Carcinoma of the Liver.**—M. F., F. M., N. S. The liver contains innumerable small or larger whitish nodules, many of which possess yellow or red and yellow centers. *Fresh examination* shows the discoloration of the centers in some of the nodules to be due to fatty changes in the tumor cells or to hemorrhage or deposits of bile and biliary pigment. Where the nodules occur on the surface of the organ these usually possess a central depression (the so-called cancer navel). The compression and derangement of the typical structure of the liver about the nodules may be observed even with the unaided vision. In sections which include the boundary of the metastasis and the adjacent liver structure, the results of the rapid growth of the nodule are apparent. This causes flattening of the adjacent liver cells (Plate XXXIV., Fig. 5), which results in atrophy and autochthonous pigmentation.

2. **Secondary Infiltrating Carcinoma of the Liver.**—In this form the hepatic tissue is infiltrated by the carcinomatous cells. No macroscopic evidence of the infiltration may be discernible. M. F., F. M., N. S. or Zenker, celloidin, Mallory's connective-tissue stain. In sections a separation of the columns of liver cells will be noted. The distance between them is not that of the normal slender capillary spaces, but wide cavities filled with rows and groups of large epithelial elements with deeply stained protoplasm and nuclei. The staining qualities of the carcinoma cells serve to readily differentiate them from the pale

PLATE XXXIV.

Fig. 1.

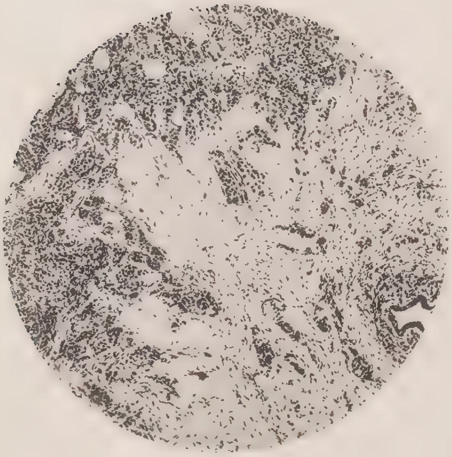


Fig. 2.

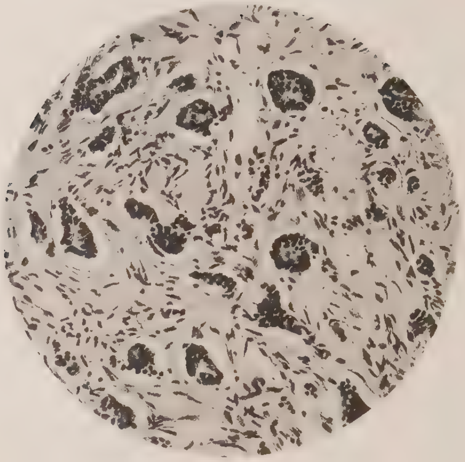


Fig. 3.

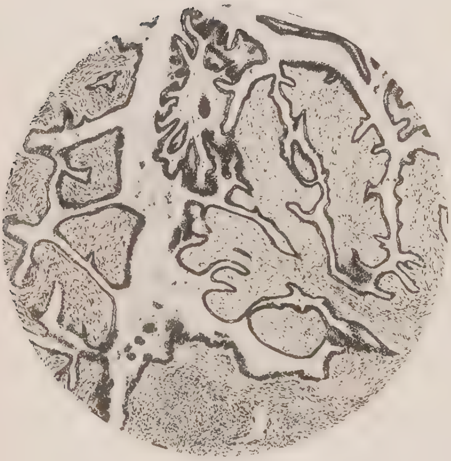


Fig. 4.

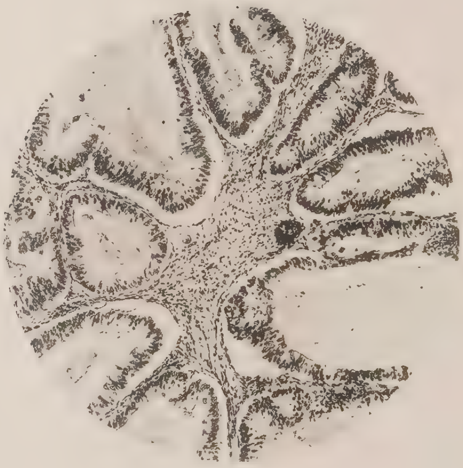


Fig. 5.

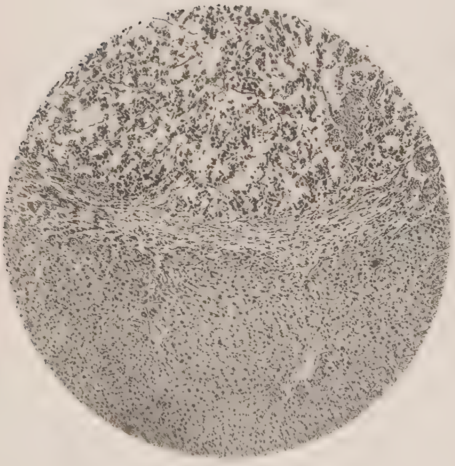
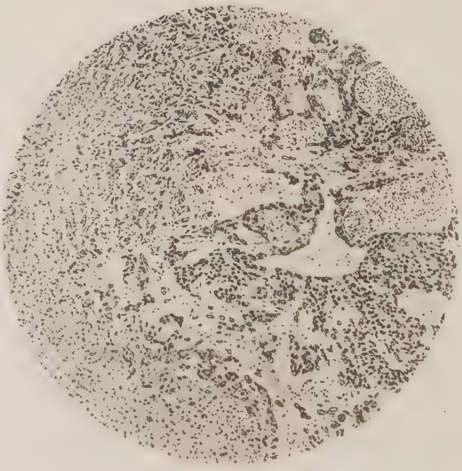


Fig. 6.



epithelium of the liver. The infiltration invariably starts from the finer branches of the portal veins, within which cancer cells are occasionally found.

17. Malignant Epithelioma of the Chorion.

One of the most interesting forms of carcinoma has its origin in the epithelial coverings of the chorionic villi. A definite relation exists between the development of these tumors and hydatidiform mole, many of them developing as late as several months after the expulsion of the mole. The growth of the tumor is likewise associated with repeated hemorrhages from the uterus which replace the regular periods of menstruation. The early diagnosis of this tumor is of great importance, as very small primary growths may lead to extensive metastases. The fragments obtained by curettement should be hardened in M. F., cut on the freezing microtome or embedded in celloidin. N. S.

The microscopic appearance of these growths is exceedingly variable. Most of them contain both of the characteristic coverings of the chorion, the so-called syncytium and the underlying layer of Langhans. Either one of these cell forms may predominate, and a few rare cases have been described where the tumor was exclusively composed of one variety. Well-developed villi with œdematous connective-tissue stroma, although without vessels (changes characteristic of hydatid mole), may be found in the tumor in which case the continuity of the epithelial coverings and the cells of the tumor may be determined. (Plate XXVII., Fig. 2.) It occasionally happens that during the course of normal pregnancy the chorionic villi become detached and are carried with the blood into the open uterine veins, but in these cases they invariably disintegrate. When the malignant growth is established the transportation of detached villi or epithelial cells is followed by metastatic tumor formation. The con-

REFERENCE:

For the more minute histology of invasion of the blood and lymph vessels by carcinoma, see *Goldman*, Beiträge zur klin. Chir., Bd. XVIII., 1897.

DESCRIPTION OF PLATE XXXIV.

FIG. 1. Soft carcinoma of the breast. See p. 256. Low power. Hæmatoxylin. The invading carcinoma to the left. The carcinoma cells arranged in small alveoli or in projecting rows (magnifier). These penetrate between the large fat cells of the mamma. To the right lacteal duct in cross section.

FIG. 2. Scirrhous mammæ. See p. 257. High power. Hæmatoxylin. The alveoli of the carcinoma cells are small and embedded in a dense stroma of connective tissue. The cells of the connective-tissue stroma spindle-shaped with spindle and oval nuclei.

FIG. 3. Non-malignant papillary cystoma of the ovary. See p. 251. Middle power. Hæmatoxylin. Papillary projections of the connective-tissue stroma covered by a simple layer of epithelium.

FIG. 4. Malignant papillary cystoma of the ovary. See p. 251. Middle power. Hæmatoxylin. The papillary projections and spaces between the papillæ lined with multiple layers of epithelium. Nuclei of varying size and form placed at different elevations in the cells. A small amount of connective-tissue stroma.

FIG. 5. Carcinoma metastasis in the liver. (Nodular form.) See p. 258. Low power. Hæmatoxylin. Above, a carcinoma nodule with fine fibrillated reticulum. Between the reticulum loosely arranged carcinoma cells. Below, the parenchyma of the liver. The layers of liver cells adjacent to the nodule show definite flattened and concentric arrangement.

FIG. 6. Chorion carcinoma, metastasis in the liver. See p. 260. Low power. Hæmatoxylin. To the right the structure of the tumor. Many of the cells show typical syncytial form surrounding open spaces, some of which contain large numbers of leucocytes. To the left the hepatic structure. With a magnifying glass the epithelial cells are found within the hepatic capillaries and may be differentiated from the pale epithelium of the liver by their deeply stained protoplasm and large irregular nuclei.

nective-tissue stroma of the villi takes no part in the development of the tumor. The fragments of the primary tumor obtained by curettement usually consist of proliferated epithelium without villi. Where the scraping includes a portion of the muscular structure the penetration of the cells of the tumor into the uterine wall may be followed. Whereas in the course of normal pregnancy only the superficial layers of the mucosa are invaded by the epithelial cells of the chorion, or these are found in the immediate neighborhood of the superficial vessels, the scrapings from the tumor reveal a most extensive infiltration of the muscular structure of the uterus by columns or masses of cells. In many places the muscular structure is split up, or where the cells are closely packed is wanting. A characteristic change in the muscular coats is the large number of thin-walled vessels which they contain. These are surrounded by closely packed chorionic epithelial cells, which in many cases break through the vessel walls and may be found within the lumenæ. The result of this invasion of the vessels is more or less extensive hemorrhages, which give to portions of the tumor a characteristic deep red coloration. The invasion of the blood vessels is followed by the early formation of metastases. The diagnosis of epithelioma of the chorion from fragments of tissue obtained by curettement is not always a simple task for the reason that fœtal tissue may be retained within the uterus for a considerable period of time and still retain their staining qualities. These remains are frequently imbedded in the uterine wall and may even be found between the bundles of muscle fibers. In hydatidiform mole the uterine structure is likewise invaded by the chorionic epithelioma. For this reason a diagnosis of malignant epithelioma of the chorion may only be made when the scrapings or tumor consist of fœtal cells, and when these contain numerous karyokinetic figures and invade the muscular structure of the uterine wall. The *fresh examination* of the tumor reveals the presence of large protoplasmatic masses containing fine fat droplets (syncytium) and smaller epithelial elements containing glycogen (cells of Langhans).

18. Suprarenal Epithelioma. (Hypernephroma.)

The suprarenal epitheliomata are tumors which are derived from misplaced suprarenal rests (Grawitz). These rests are occasionally encountered in normal kidneys directly beneath the capsule, where they appear as sharply defined flattened structures of intense yellow color and about the size of millet seeds. When examined in the fresh state they are composed of polyhedral cells which contain a large amount of fat. M. F., F. M., N. S. Microscopic sections through these small structures reveal an arrangement of cells more or less like that of the adrenal glands. They possess a delicate stroma almost entirely composed of capillaries, the meshes of which are filled with large polyhedral epithelium. Occasionally the structure is divided into two or three layers, which then resemble the zona glomerulosa, fasciculata or reticularis of the normal suprarenal. The entire structure is more or less completely separated from the kidney by a connective-tissue capsule. The cells of the rest occasionally extend through defects in the capsule and become mixed with the cells of the kidney or even formed into glandular structures closely resembling the uriniferous tubules. From these, cysts occasionally develop in the neighborhood of the rest. Recent investigation indicates that some of these glandular structures are remains of the Wolffian body.

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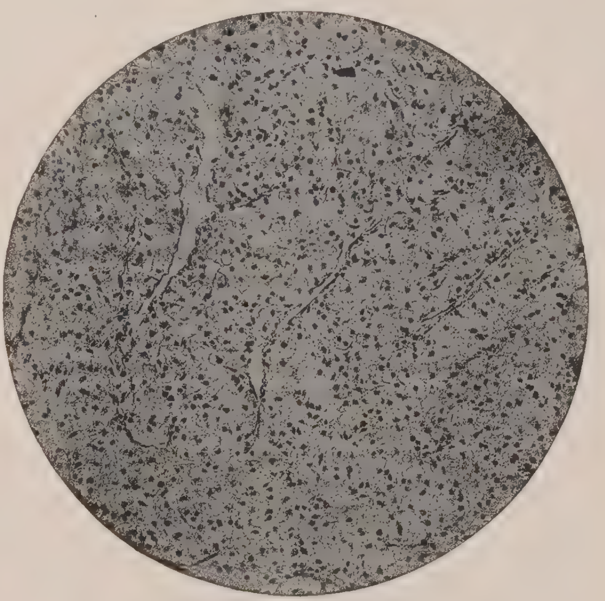
Ref. see Lubarsch-Ostertag. Ergebnisse, V., 1898.

It will be seen from this description that these small structures are remains of the adrenal glands which have become included in the kidney during the period of embryonic development. They are especially interesting as the occasional source of non-malignant and often malignant tumors. These are characterized macroscopically by an unusual yellow coloration. The incised surface of the tumor shows evidence of extensive hemorrhage and marked necrosis, which give to it an appearance very much like that of a perithelioma. *Fresh examination* reveals extensive deposits of fat and glycogen in the tumor cells. For the differentiation of the glycogen the tumor must be hardened in alcohol. The topography of the tumor and the remaining histological characteristics are best preserved in material hardened in M. F. or sublimate, F. M. or embed. N. S. Microscopically the tumor may possess a structure very much like that of the ordinary adrenal. In other cases it resembles more closely that of a perithelioma consisting of large openings lined by high cylindrical epithelium which rests directly upon the capillary structure of the stroma. Here and there where the openings of the tissue are not so large, the tumor presents an adenomatous type. More rarely portions of the tumor will present a sarcomatous appearance, produced by a close arrangement of the tumor cells which gives them a spindle form. Giant-cell formation is commonly met with. Where the derivation of the tumor from adrenal tissue cannot be followed, it is difficult to determine whether one is dealing with an ordinary perithelioma or a suprarenal epithelioma.

Fig. 38.



Hypernephroma. (Hæmatoxylin, middle power.)

19. Dermoids and Atheroma Cysts.

Cysts whose walls possess the characteristic structures of the skin are known either as dermoid or atheroma cysts. These occur most commonly in the region of the outer skin where they develop from fœtal inclusions. The contents of the dermoid cyst are usually a sebaceous material in which is embedded a large amount of hair. The contents serve to distinguish true dermoids from the so-called atheroma cysts, which are produced by cystic dilatation of the hair follicles or from the so-called simple epidermoid cysts which are likewise derived from fœtal inclusions. All of these forms of cyst contain sudoriferous material.

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Busse. Virchow's Archiv, Bd. 157, 1899.

When examined with the microscope this material consists of fatty detritus, numbers of squamous epithelial cells without nuclei and a large amount of cholesterin. The epithelial cells may be recognized by their delicate outlines and as they commonly occur in groups may produce pavement-like figures. M. F., F. M., N. S. Microscopic examination reveals the essential differences between true dermoids and the epidermoidal and simple retention cysts. The wall of the retention cysts (follicle cyst) is composed of smooth connective tissue, upon which rests a thin layer of flattened epithelium. The walls of the epidermoid cyst (these are the ordinary atheroma cysts) show the characteristic papillæ and flattened epithelium of the skin, while the dermoid cysts contain all of the characteristic structures of the skin including hair follicles, sweat and sudoriferous glands. The sudoriferous glands are usually very large, while the sweat glands occasionally show an unusual development of the spindle cells of the tunica propria.

20. Teratoma (Embryoidal Tumors).

The teratomata are tumors composed of the various structures of the human body and occur most commonly in the reproductive glands. In the ovary they are usually in the form of cysts which are more or less completely lined with typical skin, for which reason they have until recently been classed as dermoid cysts. According to the recent investigation of Wilms these tumors possess a complicated structure, in which may be recognized an elevation covered with hair, the so-called head of the tumor. This head usually contains osseous structures which may be recognized on palpation. In the neighborhood of the head may be found one or more teeth, either partly or completely formed. M. F. Decalcify, embed in celloidin, N. S. Elastic fiber stain. Neuroglia stain. Axis cylinder stain. Connective-tissue stain.

The sections should include the hairy elevations and various portions of the wall. Microscopic sections through the elevation show that the so-called head is covered with greatly thickened skin, which contains a large number of hair follicles, hypertrophic sudoriferous glands and sweat glands, with greatly thickened tunica propria. The cutis may likewise contain more or less extensive bundles of smooth muscle fibers. The subcutis is composed of fat tissue which is usually bounded by some osseous structure. In the deeper portions of the elevation are found the most variable formations, cystic cavities lined with ciliated epithelium and walls composed of muscle fibers in which are embedded masses of cartilage and which may contain mucous glands; in other words, more or less closely following the structure of the upper air passages. Other cystic structures are lined with villous projections covered with cylindrical epithelium. Embedded in the cyst wall will be found lymphoid tissue and a more or less complete circular ring of smooth muscle fibers indicating that the structure represents the intestinal tract. Lastly, cerebral substance may be found containing corporæ amylaceæ, nerve fibers and ganglion cells. The great variability and richness of these structures, among which are salivary glands and structures representing the thyroid, show that the tumor has developed from either an ovum or the closely related primordial ovum.

The teratomata of the testicles are usually of the solid variety and contain large numbers of small cystic cavities and more or less extensive deposits of cartilage. M. F., F. M., N. S. Under the microscope these tumors reveal the

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PLATE XXXV.

Fig. 1.

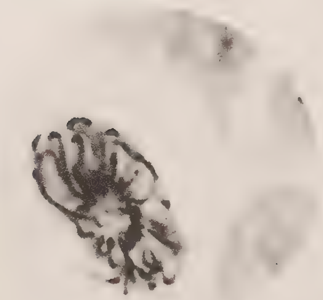


Fig. 2.

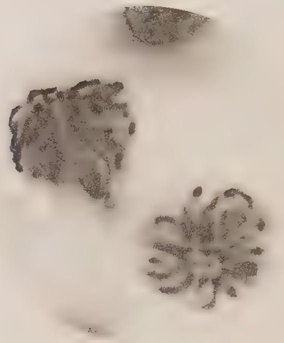


Fig. 3.

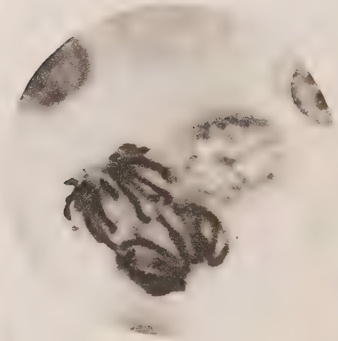


Fig. 4.

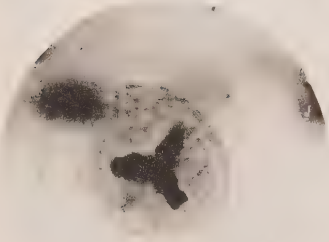


Fig. 5.

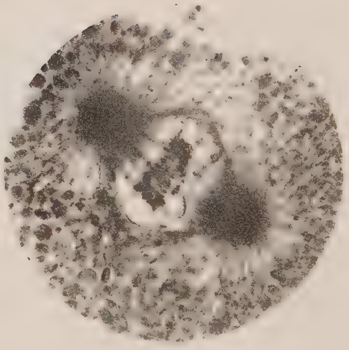
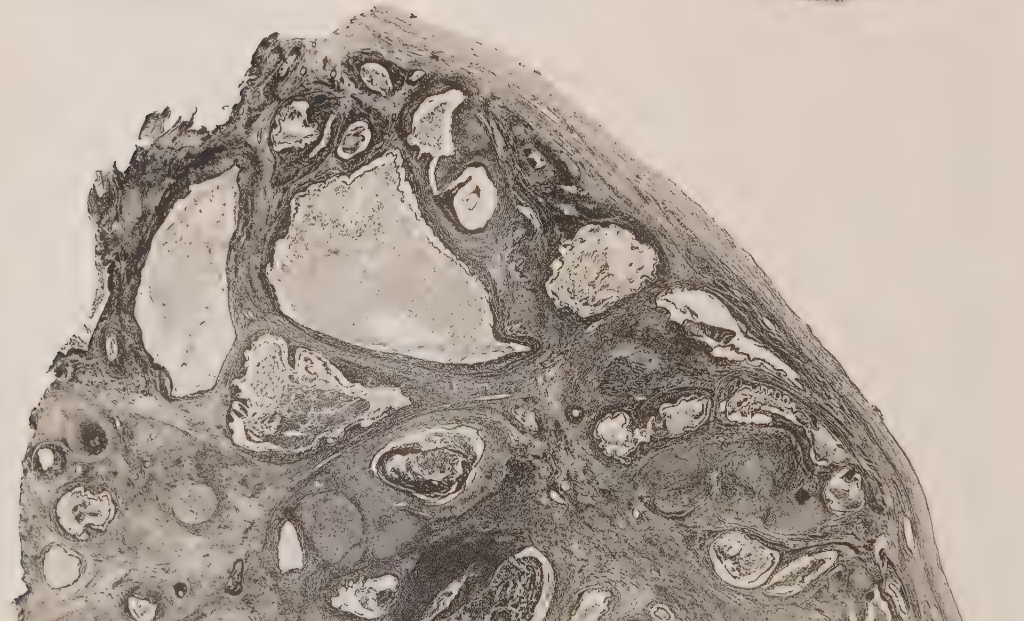


Fig. 6.



greatest variety of structure. Where the cavities are numerous an appearance very much like that of ovarial cystoma may be produced. The cartilaginous deposits may be detected by their staining and morphological characteristics. (Plate XXXV., Fig. 6.) Many of these tumors take on sarcomatous or carcinomatous change.

The Etiology of Carcinoma and Sarcoma.

If we review the different forms of tumor the question will undoubtedly arise, how are we to explain their formation and how can we account for the unusual proliferative energy which they exhibit? A short or satisfactory answer to this question cannot be given. The more recent investigations, especially those of Ribbert's pupils, have, for certain classes of tumors, strengthened the theory of Cohnheim, that many are derived from embryonic inclusions. This applies especially to the hypernephromata, some forms of myomata, neuromata and gliomata. This explanation does not, however, suffice for many varieties of tumors which develop from apparently normal tissue. If we cannot explain why non-malignant tumors develop from normal structures, then we are in still greater difficulties when we come to consider the etiological factor of malignant growths. We can make certain distinctions between non-malignant tumors which at least follow the laws necessary to the existence of the host and the malignant which do not conform to these laws. The principal distinction is in the enormous proliferative energy exhibited by the malignant form. Ribbert believes that the atypical proliferation of the cells in carcinoma is produced and preceded by inflammatory irritation of the connective tissue which causes detachment of the cells from their base without disturbing their nutrition. Once detached Ribbert believes that the cells are no longer bound by their previous environment and begin a career of reckless and unrestrained proliferation. The investigations of other observers have shown that the carcinomatous change in the epithelial cells is not always preceded by changes in the connective tissue. On the other hand, we recognize many conditions in which the epithelial cells are separated from their bases and surrounded by connective tissue without carcinoma resulting.

The best example of this isolation of epithelial elements is found in the invasion by the cells of the chórion of the muscular structures of the uterine wall in pregnancy. Many of these cells are likewise carried through the circulation and deposited in various parts of the body without leading to tumor formation. It is therefore evident that in malignant growths some factor is

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DESCRIPTION OF PLATE XXXV.

FIGS. 1–5. Normal karyokinetic figures. See p. 230.

FIG. 1. Large monaster in normal cell from tail of triton.

FIG. 2. Chromatic skein formation in the nuclei of the (tail of triton) daughter cells immediately after division.

FIG. 3. Diaster (tail of triton).

FIG. 4. Tripolar division in a carcinoma cell.

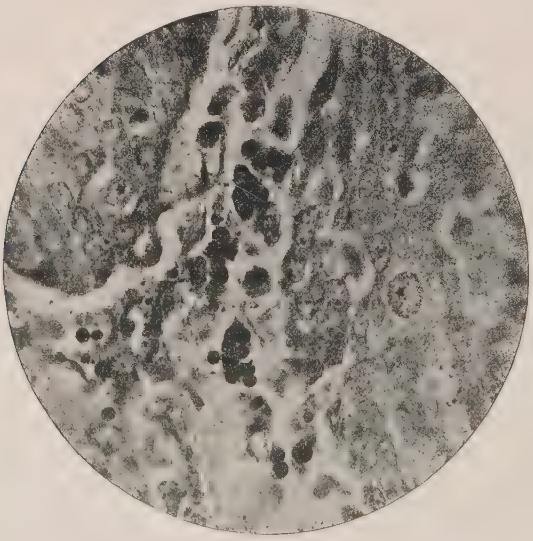
FIG. 5. Chromatic spindle formation. Between the chromatic spindles the equatorial plate. At the apices of the chromatic spindles well-defined polar radiation (from frog embryo).

FIG. 6. Teratoma of the testicle. See p. 262. Very low power. (Microcollinear.) Hæmatoxylin. The tumor is composed of a connective-tissue stroma including masses of cartilage and large cystic cavities. To the left and below deeply stained layers of cartilage. To the right and above numerous cystic cavities some of which are lined with cylindrical epithelium, others with multiple layers of squamous epithelium. About the tumor to the right a well-defined connective-tissue capsule.

introduced which is not present in normal epithelium, even when placed under the conditions which are followed by tumor formation where malignant tumors are concerned. That the agent which gives to the cell its remarkable proliferative energy, affects primarily the cell is shown by the formation of metastases from single cells, and the ability the cell possesses of retaining its malignant character when transported to widely distant regions of the body. A careful analysis of all of the current theories, except the parasitic, will reveal the fact that none offer any adequate explanation of this phenomenon. Although the parasitic theory offers the greatest hope for the ultimate solution of this complicated problem, the immediate difficulties which confront it have prevented its general acceptance. The majority of scientists have rejected it on *a priori* grounds alone. In spite of these difficulties, a certain number of those who have investigated the subject on an experimental basis have reached the conclusion that carcinoma and sarcoma are parasitic diseases, although the actual nature of the parasite has not been established. The work of Plimmer has undoubtedly established the fact that carcinoma cells contain definite structures (see Pl. XXXVa) of characteristic appearance (in specially hardened and stained tissues) which may be demonstrated in a large percentage of tumors (80 per cent. or higher, confirmed by the finding of the Boston Laboratory and the New York State Laboratory). These structures closely resemble certain stages in the development of the coccidium oriforme in the gall duct epithelium of infected rabbits and, as recently shown by Gorini, the vaccine organism in the inoculated corneas of rabbits.

These bodies also closely resemble certain stages in the development of the Plasmodiophora brassicæ Worinin, a fungus which infects cabbages. This organism and the blastomycetes which have been isolated from malignant growths have failed to produce, with the exception of Sanfelice's experiments, more than mesoblastic proliferation.

Fig. 39.



Russel's bodies at the margin of a squamous epithelioma.

The close resemblance of these bodies in cancer (first described by Virchow, in Virchow's Archiv, Vol. I., later by Thoma-Sjöbring, Jackson Clarke, and Plimmer) to certain stages of the coccidia (Podwyssozki and Sawtschenlso) and the Plasmodiophora brassicæ Worinin (Navaschine) strongly suggests their parasitic nature. The fact that they so closely resemble certain stages of both an animal and vegetable parasite is not remarkable in the light of the close resemblance which certain stages of these organisms bear each other.

There is therefore a strong presumption that the Thoma-Sjöbring bodies are of parasitic nature. This presumption has not, however, gone unchallenged in the past, nor will it likely fail to elicit criticism in the future. A number of investigators who have undoubtedly observed these bodies, have attempted to trace their derivation from the protoplasm or nuclei of the cells and explain

PLATE XXXVa.

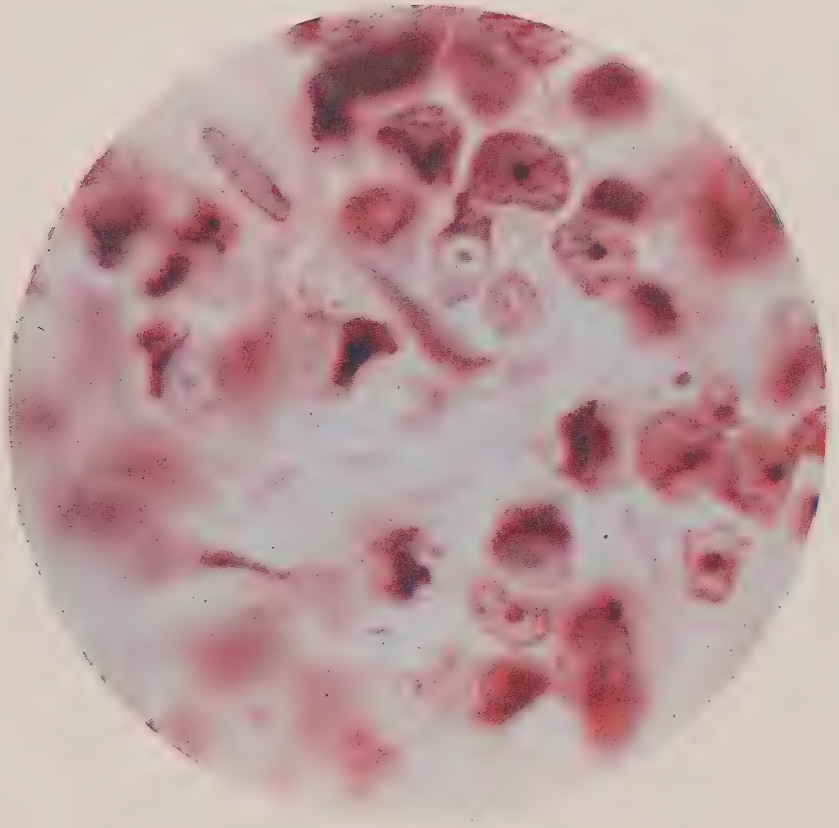
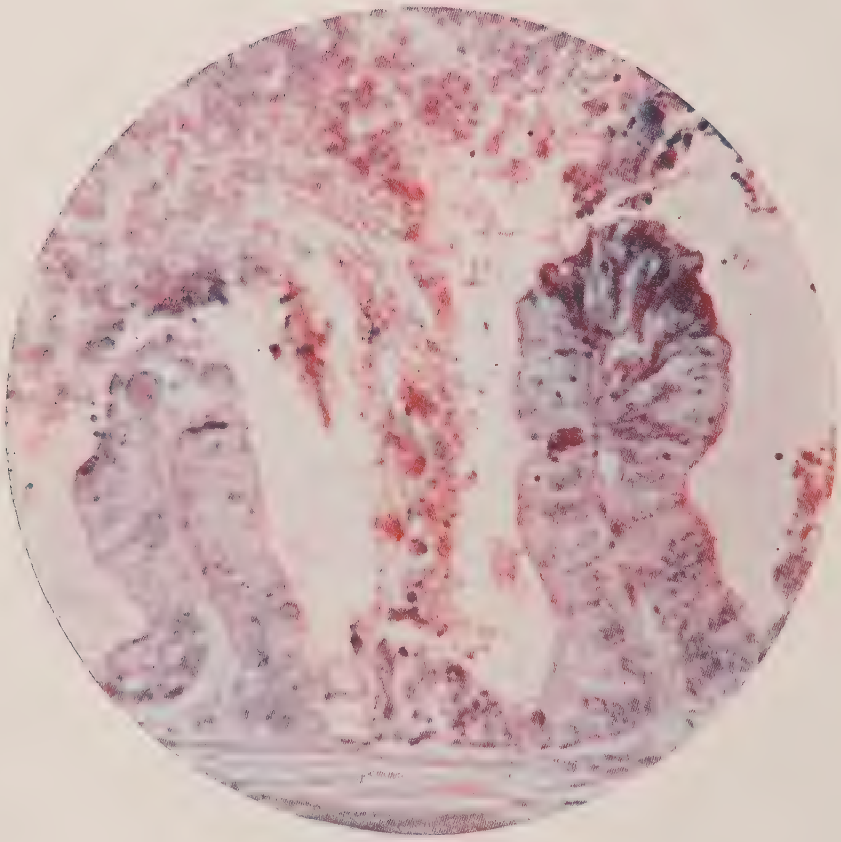


PLATE XXXVb.



them as the product of peculiar degenerative processes. A most elaborate and extensive work of this nature is that of Pianese, 1895, who concluded at that time that all of the bodies, including those of Russel (Fig. 39), Thoma-Sjöbring (Plate XXXVa), and others were the result of degeneration of the protoplasm. He likewise carefully investigated the atypical karyokinetic figures of the carcinomatous epithelium. His conclusions of that time were based upon staining methods and the examination of stained sections. That it is impossible to solve so complicated a problem in this manner is emphasized by Pianese's most recent publication (Zeitschrift f. Hygiene u. Infect. Krank., Vol. XXXVI., Heft 3) in which, after having studied an organism (protozoon) which infects the epithelium of the kidney tubules in guinea-pigs, he concludes that this organism presents an appearance in the tissue indistinguishable from the questionable bodies in the epithelium of carcinoma, and that the adjacent epithelial cells, even when uninfected, show the atypical karyokinetic figures, and other nuclear changes, supposedly characteristic of carcinoma.

Comparative evidence as to the significance of the bodies in carcinoma, is found in molluscum contagiosum (contagious epithelioma), which is considered by many observers to be a sporozoan disease. This growth consists of small nodules, seldom larger than a pea, which develop in the skin and possess a fine, central opening. On pressure the structure liberates a small amount of sudoriferous material. In cross section under low power the tumor is possessed of a lobulated structure surrounding the central opening, presenting an appearance very much as if the skin was folded into a small pocket. (Plate XXXV., Fig. 1.) The papillæ of the skin in the neighborhood of this structure are wider at the base than at the surface and penetrate somewhat more deeply than normal into the connective tissue. When examined under high power the cells lining the cavity of the tumor show no evidences of hornification. On the contrary, beginning with the middle layers of the rete Malpighi, they take on an oval form. The nuclei are pressed to one side, the protoplasm changes into a clear hyaline material and the entire structure develops a highly refractive appearance. In the middle layers a certain amount of keratohyaline granulation may be detected in these ovoid structures. Toward the surface the nuclei disappear and the bodies possess an appearance exceedingly like coccidæ.

Sjöbring, who has devoted over ten years to the investigation of carcinoma, has recently concluded that the disease is due to an infection of the epithelium by a low animal parasite which does not conform to any of the known types, but which can be isolated from carcinoma and is then capable of producing epithelial infection in animals.

Scheuller has also arrived at a similar conclusion and describes the cultivation of an organism larger and in many ways different from that of Sjöbring.

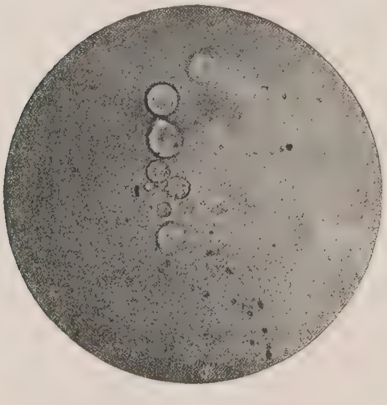
When it is remembered that the coccidia are capable of an endogenous and exogenous cycle and that the organisms in question are of an entirely unknown order is may be that observations at first apparently conflicting may be ultimately reconciled.

The occasional detection of yeast organisms in cancer has not led to the proof of a more than causal connection of these organisms with the disease. There is, however, no doubt that Sanfelice has twice succeeded in producing adenomatous tumors by the inoculation of animals with such organisms. A similar experiment has succeeded in our own hands (Gaylord), but as the animal inoculated was kept in a kennel where animals inoculated with cancer had previously been confined, and no yeast could be obtained by culture, or demonstrated in the tumor and the questionable (protozoan) bodies were present in large numbers, it seems more probable that the animal was subsequently infected in the stall, than that the tumor was due to the blastomyces.

A long series of attempts (with this single exception) to produce adenomatous tumors with yeast organisms have proved fruitless in our hands and those of others, the lesions being granulomata of mesoblastic origin in which the yeasts could be readily demonstrated. In a work of this nature it is impossible for us to enter fully into the work of one of us (Gaylord), further than to state that his experiments are practically confirmatory of those of Sjöbring, and that he has found that the bodies described by Russel, and those of Thoma-Sjöbring are practically constant in carcinoma.

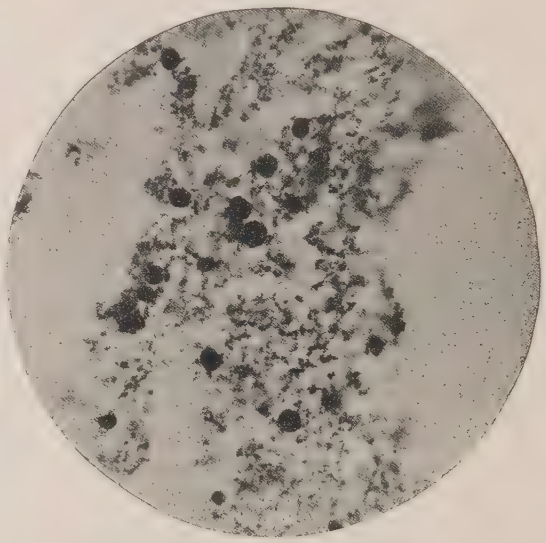
He likewise observed in fresh cancer and peritoneal fluid certain hyaline bodies (Fig. 40) which could be fixed and stained (Fig. 41), although with difficulty. These conformed when stained very closely with Russel's bodies (Figs. 38 and 43), and it was found that when cancerous peritoneal fluid containing them was injected into the peritoneal cavities of guinea-pigs, these bodies appeared to increase in number and varying forms, including groups of bodies resembling spore sacs with a delicate membrane could be found. The smaller bodies were also observed under the microscope to change form and the development of the small spherical bodies into those of larger size and irregular shape, and ultimately into groups of spherical bodies like the original body, was followed. These bodies, except the small spherical bodies, do not resemble very closely the bodies found in the hardened and stained tissue, but in case they are of protozoan nature this is to be expected. The observer is inclined to believe that these changes do not include the complete cycle of the organism.

Fig. 40.



Hyaline bodies in the fresh state. (Oil immersion.)

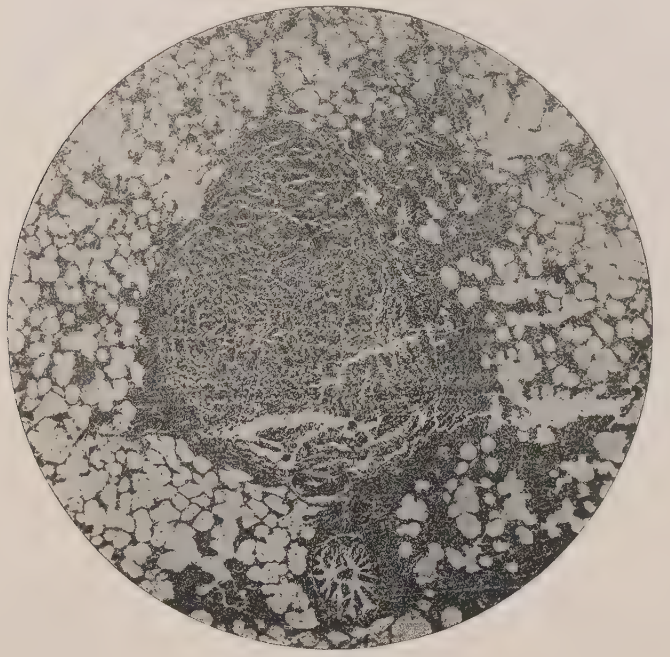
Fig. 41.



Hyaline bodies in cancerous, peritoneal fluid hardened and stained according to Plimmer. (Oil immersion.)

Having observed the presence of these bodies, the fluids which contained them were injected into a large number of animals; in five of these epithelial changes were noted which in the larger nodules conform in structure and appearance (Fig. 42), as well as by the presence of the questionable bodies to carcinoma. In a large number of animals no epithelial changes were found, but the bodies (Russel's) could be found in the tissues, especially lymph nodes and spleen. The criticism has been offered that epithelium could not be excluded from peritoneal fluid containing these bodies, and that therefore the epithelial prolifera-

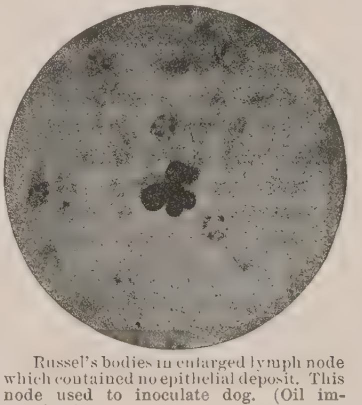
Fig. 42.



Carcinomatous nodule in the lung of a guinea-pig which had been inoculated with cancerous peritoneal fluid from man containing hyaline bodies. (Low power.)

tion might be the result of transplantation of carcinomatous epithelium. If this is the case, then we must conceive of a transformation of the injected epithelium into that of the animal, as the derivation of the nodules could be traced to the normal epithelium of the animal. The questionable bodies were the only constant factor in the experiments. In one animal (an as yet unpublished experiment) a large lymphoma of the spleen resulted from inoculating the animal in the jugular with a lymph node which had been removed by operation from the neighborhood of a cancer of the breast. This node contained no epithelial deposits, but large numbers of Russel's bodies, usually in the lymph sinuses or at the margins of the follicles. (Fig. 43.) Cultures showed that the node was free from bacteria. It was preserved in a sterile petri dish and kept at body temperature until thoroughly dry, when it was powdered in a sterile mortar, mixed with sterile salt solution and a portion was injected in the jugular of a healthy dog. Ninety days after, when the animal was killed, a tumor as large as a walnut was found on the lower margin of the spleen. This was continuous with the organ and proved to be the result of hyperplasia of the splenic elements.

Fig. 43.



Russel's bodies in enlarged lymph node which contained no epithelial deposit. This node used to inoculate dog. (Oil immersion.)

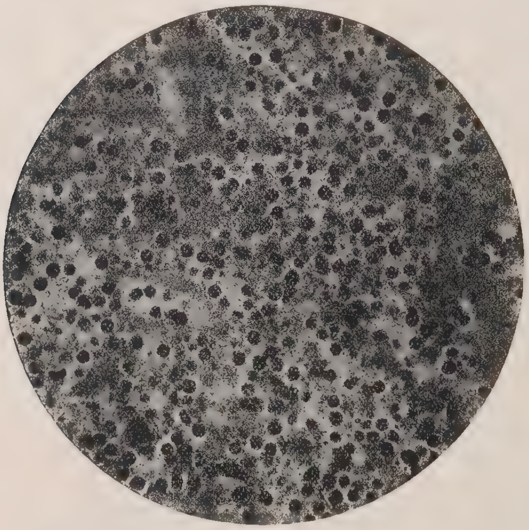
Sections of tissue hardened and stained according to Plimmer's method revealed the presence in the tumor formation of great numbers of bodies (Fig. 44) identical in appearance and staining qualities with those in the lymph node.

This would indicate that these bodies have an identity, and bear a definite relation to the proliferation of the cells, in this case those of the spleen. The enlargement of the regional lymph nodes near carcinoma is familiar, and in these Russel's bodies have frequently been found.

The similarity of these bodies in the hardened section and those of the vaccine organism has already been noted. The recent observations of Calmette, Copeman, and Funck indicate that the bodies observed by them present great similarity of appearance to those described by Gaylord.

In malignant lymphoma and in the terminal stages of cancer G. found similar bodies in the blood and in the organs after death. It is his opinion that these bodies are of protozoan nature and will prove to be the cause of the processes in which they are found.

FIG. 44.



Field of Russel's bodies from lymphomatous tumor of spleen. Dog inoculated with lymph node shown in Fig. 42. (Oil immersion.)

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XVIII. BLOOD.

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The blood, as a tissue, is so frail and perishable that its study in pathological processes is best pursued on specimens taken *intra vitam* from the sick patient. Hence the pathology of the blood has become in large measure a clinical study and one so technical and extensive as to require its partial separation from general pathology into a "specialty," called hæmatology. The consideration of the blood in these pages will therefore be undertaken chiefly from the point of view of clinical pathology.

The clinical study of the blood as an aid to diagnosis and prognosis has in late years assumed an important rôle in general medicine and surgery and seems destined soon to become as much a routine practice as urinalysis has long been.

In some few diseases the condition of the blood is pathognomonic and diagnostic, in many more conditions it is symptomatic and furnishes valuable suggestions that direct attention toward the real nature of the disease, while in other morbid processes the blood sheds no light on the problem of diagnosis. It is important to emphasize the fact that the condition of the blood is usually merely one of many symptoms in disease and its interpretation must be made only in connection with all the other symptoms and physical signs, which of course demands that the hæmatologist be not a mere blood worker, a kind of specialized pathologist, as it were, but a thorough and competent clinician, as well, capable of weighing and interpreting the evidence furnished by the blood in relation to all the other signs.

In a brief chapter devoted to this subject we may not attempt to more than outline the more important principles and facts and to briefly emphasize the salient points of practical value. For a more comprehensive exposition of the subject the student is referred to special treatises, of which may be recommended the following in English, German and French.

The Formed Elements of the Blood.

The chief morphological elements of the fresh blood consist of the *red corpuscles*, the *white corpuscles* or *leucocytes*, and the *blood platelets*, to which may be added the *blood dust* (*hæmoconien*) of Müller, and the *fibrin*, which latter is formed after a few minutes' standing.

The **Red Corpuscles** or **Erythrocytes** (see Plate XXXVI.) are biconcave disks, pale yellow in color, measuring 7.5μ in diameter, and numbering about 5,000,000 per cubic millimeter in normal blood for men, and 4,500,000 for women. In morbid conditions the red corpuscles may vary from the normal in number, size, form and color, and may exceptionally be nucleated. In conditions causing concentration of the blood, such as stasis, profuse diarrhœa, etc., the red corpuscles are of course increased in number (polycythæmia), while in conditions of temporary dilution, as from the ingestion of a large quantity of fluid, the red corpuscles are reduced below the average in number (oligocy-

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Ewing. Clinical Pathology of the Blood. Philadelphia and New York, 1901.

Ehrlich and *Lazarus.* Die Anaemia. Wien, part I., 1898, part II., 1900 (being heft I. and II., theil I., band VIII., of Nothnagel's Spec. Path. und Ther.).

v. Limbeck. Grundriss einer klinischen Pathologie des Blutes. 2d ed., Jena, 1896.

Schmaltz. Die Pathologie des Blutes und der Blutkrankheiten. Leipzig, 1896.

Hayem. Du Sang. Paris, 1889.

thæmia). Variations in the size, shape and color occur in severe anæmias, and in certain anæmic conditions nucleated red corpuscles are found. The red corpuscles stain with acid dyes. The terms applied to the different types and conditions of the red corpuscles are as follows:

Erythrocytes.—Red cells, in general.

Normocytes.—Red corpuscles of normal size (7.5μ).

Microcytes.—Small red corpuscles (3–5μ).

Megalocytes (macrocytes).—Large red corpuscles (10–15μ).

PLATE XXXVI.

(*Stained with the Ehrlich triple stain.*)

FIG. 1. TYPES OF LEUCOCYTES. *a.* (Polymorphonuclear) neutrophile—nucleus twisted and irregularly stained in blue; protoplasmic granules fine and violet in color. *b.* (Polymorphonuclear) eosinophile—the nucleus, blue, is single, but so contorted that it appears on the surface as three distinct spots; protoplasmic granules coarse, round and stained a bright red. *c.* Myelocyte (neutrophilic)—nucleus large, oval, eccentric, pale blue; granules as in *a*; the cell is larger than the polymorphonuclear neutrophile and differs from it otherwise in the large, round, oval, or slightly indented nucleus. *d.* Eosinophilic myelocyte—similar to *b* except in its large, pale, round nucleus; note the tendency of the granules to separate and spread. *e.* Large mononuclear leucocyte or large lymphocyte—the nucleus large, eccentric, pale, indented; protoplasm homogeneous, relatively large in amount, and taking a faint pinkish or grayish tint. The nucleus of this type of leucocyte is usually round or oval, and when indented, as shown, the cell is sometimes called a "transitional." *f.* Small mononuclear leucocyte or (small) lymphocyte—the smallest type of leucocyte; nucleus round and very dark blue in color; protoplasm very small in amount, forming a pale, faintly stained, narrow zone about the nucleus. The rim of protoplasm is often invisible.

FIG. 2. NORMAL BLOOD. A single neutrophile occurs in a field of normal reds. Of course, not every field of this size in normal blood would contain a leucocyte.

FIG. 3. SECONDARY ANÆMIA (POST-OPERATIVE). The reds are reduced in number (oligocythæmia) and deficient in hæmoglobin (oligochromæmia), and show slight irregularity of form (poikilocytosis). One nucleated red corpuscle (normoblast), two neutrophiles and one small lymphocyte are seen. The blood represents a well-defined post-hemorrhagic anæmia with leucocytosis.

FIG. 4. LEUCOCYTOSIS, INFLAMMATORY. The reds are normal. A marked leucocytosis is represented by five neutrophiles and one small lymphocyte, the nucleus of the latter being indented and less strongly stained than usual. This illustration may also serve the purpose of showing the leucocytosis of malignant tumor (not usually so marked as here represented) and the general blood picture, except that the reds in advanced malignant tumor show a secondary anæmia, as in Fig. 3.

FIG. 5. EOSINOPHILIA, IN TRICHINOSIS. The reds are normal. A marked leucocytosis is shown, consisting of five eosinophiles and one neutrophile.

FIG. 6. LYMPHATIC LEUKÆMIA. Slight oligocythæmia. A marked increase of the leucocytes is shown, made up chiefly of lymphocytes (lymphocytosis), of which the small variety predominates. Note the variation in size and staining of the lymphocytes, a feature commonly found in this disease.

FIG. 7. SPLENO-MYELOGENOUS LEUKÆMIA. The reds show a moderate secondary anæmia, with oligocythæmia, oligochromæmia, poikilocytosis and nucleated reds (two normoblasts). The leucocytosis is massive. Twenty leucocytes are seen, consisting of nine neutrophiles, seven (neutrophilic) myelocytes, one eosinophilic myelocyte (at the lower edge of the field), two small lymphocytes and one eosinophile (polymorphonuclear). Note the polymorphous condition of the leucocytes, *i. e.*, their variation from the typical in size and form.

FIG. 8. TYPES OF RED CORPUSCLES. *a.* Normal red corpuscle (normocyte). *b–i.* Anæmic red corpuscles, of which *b* and *d–g* are poikilocytes, *i. e.*, irregularly shaped. *h.* Microcyte, abnormally small red corpuscle. *i.* Megalocyte (macrocyte) abnormally large corpuscle. *j–n.* Nucleated red corpuscles (erythroblasts), of which *j* and *k* are normoblasts, *l*, a microblast, and *m* and *n* megaloblasts, the latter with degenerated broken nucleus. The nuclei stain a deep, greenish black.

PLATE XXXVI.

Fig. I

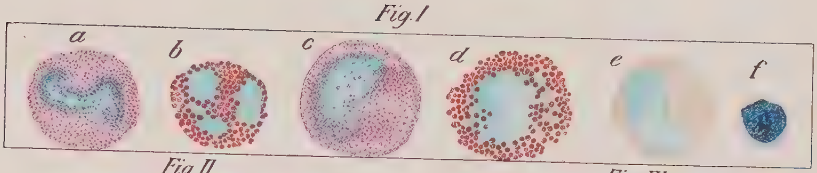


Fig II.

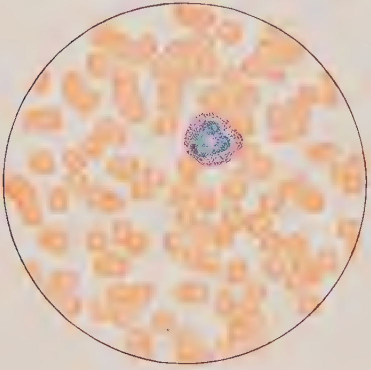


Fig. III

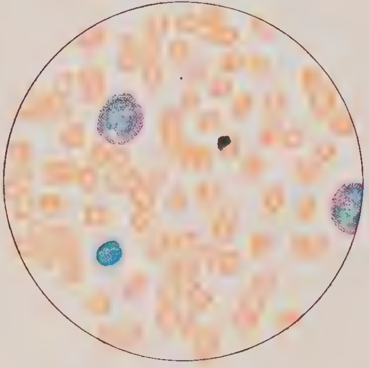


Fig IV.

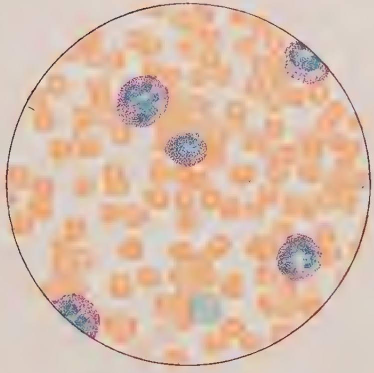


Fig. V.

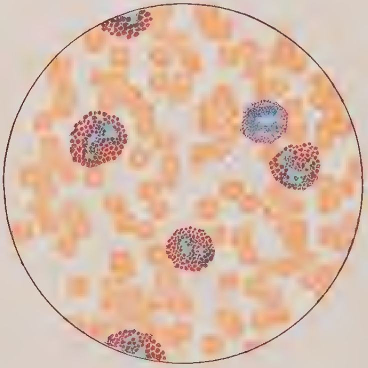


Fig. VI.

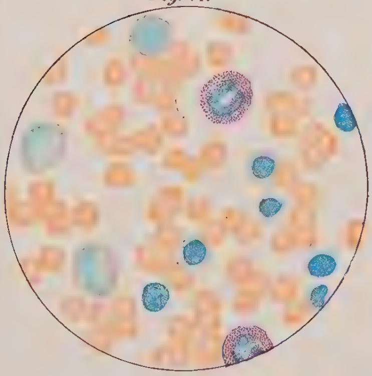


Fig. VII.

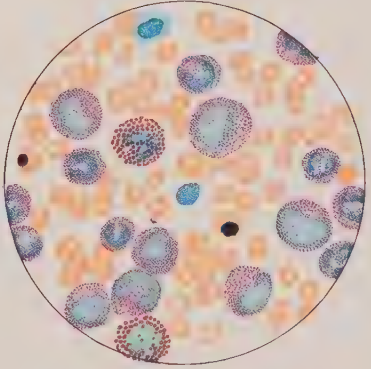
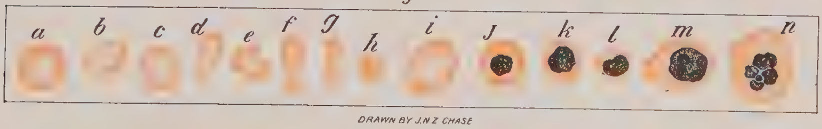


Fig. VIII.



DRAWN BY J.N.Z. CHASE

Poikilocytes.—Irregular forms of various shapes, deformed corpuscles, without reference to size. *Poikilocytosis* is said to exist in the blood when these forms occur.

Erythroblasts.—Nucleated red corpuscles, in general.

Normoblasts.—Nucleated red corpuscles of normal size (7.5μ).

Microblasts.—Nucleated red corpuscles of small size (3–5μ).

Megaloblasts (*gigantoblasts*).—Nucleated red corpuscles of large size (10–20μ).

Hydræmia.—An increase in the watery constituents of the blood, by which the blood is diluted and the corpuscles are relatively diminished in number (a temporary phenomenon).

Anæmia.—A reduction in corpuscular substance, *i. e.*, the number of corpuscles, the hæmoglobin, or both (usually).

Oligæmia.—A reduction in the amount of the blood as a whole, without reference to altered proportions of the different constituents.

Polycythæmia.—A condition of increase in the number of the corpuscles.

Oligocythæmia.—A condition of decrease in the number of the corpuscles.

Oligochromæmia.—A condition of decrease in the coloring matter (hæmoglobin) of the red corpuscles.

Polychromatophilia.—A condition of the staining quality of the red corpuscles, in which the substance stains with Ehrlich's mixture various irregular shades of brown, purple, gray, etc., seen typically in pernicious anæmia and characteristic of severe anæmia.

Granular Degeneration.—This is a term used by Grawitz to describe certain *basophilic granules* found in the red corpuscles in anæmia and toxic conditions. [These granules are stained with basic stains, such as methylene-blue and hæmatoxylin, and are said by Grawitz to be present in considerable numbers in the following affections: chronic lead poisoning (always), malaria, pernicious anæmia, carcinoma (especially when the location of the tumor leads to absorption of the toxic products of the growth), and sepsis. They are said to be absent in chlorosis, tuberculosis (pure), syphilis (all stages and even after mercurialization), chronic nephritis, chronic liver disease, diabetes, typhoid fever, diphtheria, and the acute infectious diseases. Lazarus does not regard these basophilic granules of the red corpuscles, "*punktierte erythrocyten*," as a kind of polychromatophilia, to which, however, they bear a strong resemblance.]

Crenation.—Shriveling of the red corpuscles, producing spicules over the surface, a necrobiotic process occurring upon exposure of the corpuscles to the influence of various artificial agents, *e. g.*, air, chemicals, etc. The process is without clinical significance.

The **Leucocytes** or **White Corpuscles** (see Plate XXXVI.) number usually from 5,000 to 7,000 per cubic millimeter of blood, and rise or fall in various normal and morbid processes. The upper limit of normal is usually placed at 10,000 per cubic millimeter, a figure which is more or less arbitrarily chosen, but one which is sufficiently high to include the usual variations of healthy adults. In infants and young children the normal number of leucocytes is much above this limit. The leucocytes in fresh blood are nucleated, white in color, and vary in size and appearance according to the kind of leucocyte, *i. e.*, neutrophile, eosinophile, small lymphocyte, large lymphocyte, etc. The variations in the size of the leucocytes, the form of the nucleus, and the character of the protoplasm are best studied in dried and stained specimens. Still by experience with the fresh blood these types of leucocytes can be distinguished.

REFERENCE:

Grawitz. Granular Degeneration of the Erythrocytes, and its Significance in Clinical Pathology. Amer. Jour. Med. Sci., Sept., 1900, p. 277.

The form of the nucleus, round or polymorphous, can be determined and the differences in the protoplasm, whether homogeneous, finely granular, coarsely granular and highly refractile, can be made out and the types of leucocytes thus counted proportionally. In morbid processes the differential counting of the leucocytes is important as offering clews to the nature of the process. The terminology applied to leucocytes is as follows:

Leucocytosis.—A term usually used to indicate an increase in the number of the leucocytes above the normal limit, which is usually reckoned for adults at 10,000 per cubic millimeter. Some use the term leucocytosis to indicate the number of the leucocytes in a general way without implying necessarily an increase, and employ the following two terms to indicate an increase or a decrease from the normal (Goldscheider).

Hyperleucocytosis.—An increase of the leucocytes above normal, *i. e.*, above 10,000.

Hypoleucocytosis (*Leucopenia*).—A subnormal leucocyte count, *i. e.*, below 5,000.

Polymorphonuclear (*Polynuclear*) *leucocytes.*—Those varieties (neutrophiles and eosinophiles) containing each a single nucleus of irregular shape, twisted, contorted, and resembling the forms of various alphabetic letters, viz., S, M, Z, etc.

Mononuclear Leucocytes.—Those forms containing a single nucleus of regular form, usually round, oval, or slightly indented (bean-shaped). The term is applied technically to only the small and large lymphocytes, though it might properly be applied as well to the myelocytes.

Acidophilic Leucocytes.—Referring to the chemical affinity of the protoplasm of certain leucocytes for acid stains; also called *eosinophilic* because the protoplasmic granules stain with eosin, an acid stain very commonly used; the class includes the polymorphonuclear eosinophile and the eosinophilic myelocyte.

Basophilic Leucocytes.—Leucocytes whose protoplasmic granules stain with basic dyes; such leucocytes are called *basophiles*, *mast-cells*, *mastzellen*, and are found in normal and pathological blood. Basophilic granules are frequently situated about the periphery of the nuclei of leucocytes (*Neusser's perinuclear basophilic granules*).

Neutrophilic Leucocytes.—Those whose protoplasmic granules stain with a neutral mixture of acid and basic dyes. The class includes the polymorphonuclear neutrophile and the (mononuclear) neutrophilic myelocyte. The term *neutrophile* is limited technically to the former.

Neutrophiles.—The polymorphonuclear neutrophilic leucocytes (12–15 μ, average 13.5 μ), containing a bizarre-shaped, twisted, polymorphous nucleus and a finely granular protoplasm, which stains with a neutral mixture of dyes.

Eosinophiles.—The polymorphonuclear eosinophilic leucocytes (10–16 μ, average 12.5 μ), containing a contorted nucleus and very large, round, highly refractive, protoplasmic granules, which stain with acid colors such as eosin. The granules are loosely arranged about the nucleus and tend to separate and spread apart. The term *eosinophilia* is used to denote an increase of eosinophiles in the blood.

Lymphocytes.—The *small* and *large lymphocytes* or *mononuclear leucocytes*. By some the term is restricted to the small variety, the large kind being called "*large mononuclear leucocytes.*" The small variety (8–12 μ, average 10 μ) is almost filled with a round nucleus with only a narrow rim of homogeneous protoplasm surrounding it. The large kind (12–18 μ, average 13 μ) has a large pale nucleus, round or oval, eccentrically placed, which is surrounded by a fairly large area of homogeneous protoplasm. The nucleus of the large lymphocyte is sometimes indented or bean-shaped, and the protoplasm occasionally shows suggestions of fine neutrophilic granulation, such irregular forms being called *transitionals, i. e.*, forms believed to represent transitional stages of development from lymphocytes toward polymorphonuclear neutrophiles. Intermediate forms of the lymphocytes, standing between the typical small and large lymphocyte, are frequent, especially in pathological leucocytosis, but such forms are not to be confounded with the so-called *transitional*. *Lymphocytosis* is a term used to denote a relatively high percentage of lymphocytes of either or both varieties.

Transitional Leucocytes.—See *Lymphocytes.*

Myelocytes, Marrow-cells, Markzellen.—Large cells (12–22 μ, average 16 μ) containing a single, large, round or oval, pale nucleus, eccentrically placed, and fine neutrophilic granules of protoplasm. This cell differs from the ordinary polymorphonuclear neutrophile in its greater average size and in its large, pale, homogeneous, round nucleus. The cell is supposed to be derived from the bone marrow and is seen in large numbers in myelogenous leukæmia, and in small numbers occasionally in severe anæmias.

Basophiles, Mast-cells, Mastzellen.—See *Basophilic leucocytes.*

Phagocytes.—Leucocytes that act as scavengers by devouring and destroying noxious elements in the blood and tissues (Metschnikoff). The term *phagocytosis* is applied to this process. Various offending elements, such as micro-organisms, pigment granules, etc., are thus picked out of the blood and tissues and destroyed. Phagocytes containing pigment granules are called *melaniferous leucocytes,* seen chiefly in malaria, and the term *melanæmia* is applied to such blood. Leucocytes may also contain fat globules, glycogen granules, etc., which may be revealed by proper staining methods.

The Differential Leucocyte Count.—The leucocytes are represented in the normal adult blood in the following proportions :

Polymorphonuclear neutrophiles,	60–75 per cent.	(average, 70 per cent.)
Small lymphocytes,	20–30 per cent.	(“ 22 per cent.)
Large “	4– 8 per cent.	(“ 6 per cent.)
Eosinophiles (polymorphonuclear),	$\frac{1}{4}$– 4 per cent.	(“ 2 per cent.)
Mast-cells,	$\frac{1}{50}$–$\frac{1}{2}$ per cent.	(“ rare.)

The **Blood Plaques, Plates, Platelets,** the so-called **third** corpuscle, are small, round or irregular, colorless bodies (1.5–3.5 μ), found in the normal blood in the proportion of 180,000–256,000 per cubic millimeter (van Emden, Lazarus), though some writers (Osler, Cabot, etc.) give higher figures. They are best seen in fresh blood which has been allowed to flow between two glass slips immediately from the puncture without exposure to the air. They are increased or diminished in various morbid processes, but so little is known about them that their study does not play an important rôle in clinical pathology.

The **Blood Dust,** of Müller (“ **Hæmoconien,**” “ **Blutstaubchen** ”), must not be confused with the blood platelets. The blood dust consists of minute, highly refractive, dancing, colorless granules ($\frac{1}{4}$–1 μ), that are found free in the plasma of the blood in the fresh preparation. Unlike the larger blood platelets they take no part in the process of coagulation. They are supposed to be the extruded granules of the polymorphonuclear leucocytes (Stokes and Wegefarth ; Nicholls).

Fibrin is deposited from the blood after its removal from the body and exposure to changed environment for a few minutes. It appears as a network of fine fibrils associated with collections of blood platelets, especially about the leucocytes. Its amount is approximately estimated for clinical purposes by observing the closeness of the network between the corpuscles.

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Müller. Cent. f. allg. Path., VIII., 1896.
Stokes and Wegefarth. Johns Hopkins Hospital Bulletin, December, 1897.
Nicholls. Phil. Med. Journ., 1898, February 26.

Methods and Technique of Blood Examination.

The clinical study of the blood consists of the following points of observation:

I. The Fresh Blood Examination.
II. The Estimation of Hæmoglobin.
III. Counting the Red Corpuscles.
IV. Counting the Leucocytes.
V. The Dried and Stained Blood Smear.
VI. Determining the Coagulation Time.
VII. Bacteriological Cultures.
VIII. The Widal Agglutination Reaction.

Preparation.—These various points of examination may all (except VII.) be made from a few drops of blood obtained from a single puncture of the lobe of the ear. The ear is selected by preference because it is relatively insensitive, soft and pliable, vascular, and removed from the observation of the patient. Caution should be enjoined, however, to use the finger or toe for puncture in case of local obstruction producing stasis in the vessels of the ear. The ear is prepared by gently washing it with water, followed by alcohol. If the ear is so dirty as to require considerable scrubbing, the examination should be deferred for some minutes after preparing it, lest the friction and massage of the ear should have caused local concentration of the blood, which would render the counting inaccurate. The application of alcohol must be made quickly, as its continued action is refrigerant and causes concentration. Ether should never be used on the skin as its action in cooling is very rapid and produces constriction of the vessels and concentration of blood. (See, below, Physiology of the Blood.)

The slides, cover-slips, etc., should be made scrupulously clean immediately before using by washing successively in water, alcohol, and ether, and thoroughly drying.

I. The Fresh Blood Examination.

A very small drop of blood is obtained on a cover-slip, which is immediately reversed on a slide, permitting the blood to spread itself out in a thin, even layer. No pressure should be applied to the slip. The examination of this specimen should be made within a few minutes, before drying has injured it. To the busy practitioner this examination is often sufficient to furnish a fairly comprehensive understanding of the general condition of the blood, and to the technical hæmatologist the examination of the fresh blood is of primary importance by affording information not otherwise obtainable and by affording a control over possible errors of counting. The chief points to be observed are the following: (*a*) *The condition of the red corpuscles—form, size, color, etc.* The amount of hæmoglobin can be roughly estimated from the color of the corpuscles, poikilocytosis and variations in the size of the red corpuscles are best detected in the fresh blood, and the presence or absence of rouleaux formation is noted. (*b*) *The relative frequence of leucocytes and the varieties of leucocytes.* With experience a fairly accurate estimation of the number of the leucocytes can be made from the fresh blood and thus an important control can be held over the subsequent counting. To a certain extent, also, by observing the form of the nuclei and the nature of the protoplasm the different types of leucocytes can be distinguished. (*c*) *The number of blood-platelets* can be estimated. (*d*) *The extent of fibrin formation.* This can be determined only in the fresh blood after standing. (*e*) *Parasites.* The *malarial parasites* are studied to the best advantage in the fresh blood, as finer distinctions can be drawn than in stained specimens, and the further important advantage is offered of observing amœboid movements and the motility of the pigment granules. Other parasites, as the *Filaria sanguinis hominis,* are studied to advantage in the fresh blood.

II. The Estimation of Hæmoglobin.

The various instruments employed for the clinical estimation of hæmoglobin are all of practical value by giving approximate measurements, but none are wholly satisfactory and accurate. The *von Fleischl hæmometer* is the instrument in most general use. A practical point of value to be mentioned in the employment of this instrument is the use of *distilled and carbonated water* in diluting the blood, which prevents the formation of the opacity so provokingly common when ordinary water is used to dissolve and dilute the blood for examination. Another point of technique that the writer has used with value is the polishing of the interior of the capillary pipette, after and just before using, by passing through it a fine needle threaded with soft embroidery silk. The use of this simple measure will be found to entirely remove the tendency of the blood to clog and obstruct the pipette, and the pipette will fill quickly and surely in almost every case. It need hardly be said that at least two tests should be made in every case with this instrument, as errors are very common and the first test should be controlled by repeating it. *Miescher's hæmoglobinometer* is a modification of the Fleischl instrument, designed to overcome many of its faults, and is a distinct improvement upon it. It is the *most accurate hæmoglobinometer at present obtainable,* and gives very satisfactory results. The *Gower's hæmoglobinometer,* once much in use, is now largely supplanted by other instruments. *Oliver's hæmoglobinometer* is one of the newer instruments and has received high praise, as well as criticism. In some respects it is more accurate than the Fleischl instrument, but it is less simple to use, and it seems doubtful whether it will recommend itself to the requirements of the general worker. *Tallquist's hæmoglobin scale* has recently come into use. Of all the devices for estimating hæmoglobin clinically it is the simplest and least expensive. Its principle is the comparison of the color of filter paper stained with a drop of blood with a color scale printed on paper. The method is so simple and the results of the few observations that have been made with it have been so fair, compared with the more elaborate methods, that its trial is recommended to all busy practitioners who need a quick and ready method of observation. The newest hæmoglobinometer is that devised by *Dare.* It differs from most other instruments in the fact that undiluted blood is used to match the standard color scale. This instrument, as described, is simple in construction and use and theoretically recommends itself for trial. The *Hammerschlag specific gravity method* of determining hæmoglobin is an indirect but useful one, and is growing in favor.

III. Counting the Red Corpuscles.

The *Thoma-Zeiss hæmocytometer* is the most accurate instrument in use. The blood is diluted in a graduated pipette in the proportion of 1 : 100 or, better, 1 : 200 of a mixing fluid of one of the following formulas :

(*a*) *Toisson's solution :*

Methyl violet, 5 B,	0.025
Sodium chloride,	1.000
Sodium sulphate,	8.000
Glycerin (neutral),	30.000
Distilled water,	160.000

Reference.

Dare. A new Hæmoglobinometer for the Examination of Undiluted Blood. Phil. Med. Journ., Sept. 22, 1900, p. 557.

(*b*) *Hayem's fluid:*

Bichloride of mercury,	0.5
Sodium sulphate,	5.0
Sodium chloride,	1.0
Distilled water,	200.0

(*c*) *Pacini's fluid:*

Bichloride of mercury,	2.0
Sodium chloride,	4.0
Glycerin (neutral),	26.0
Distilled water,	226.0

These solutions should all be filtered before using, as they become contaminated with yeasts and fungi, which may be mistaken for corpuscles. Toisson's solution, by coloring the leucocytes violet, enables them to be distinguished and counted in the same field with the reds, and is therefore preferred. The diluted blood is then placed upon the counter or grating and counted.

Oliver's tintometer has recently come into use for estimating the number of the red corpuscles without counting them. We have had no experience with this instrument.

The *Daland hæmatocrit* is of value for quickly estimating the number of red corpuscles by bulk after centrifuging the blood, and gives fair results in general, but for accurate results no short cut is reliable, and only the actual counting can be relied upon.

The Thoma-Zeiss pipettes should be blown out and cleaned successively with water, alcohol, and ether immediately after using, after which they will always be in readiness for use and will give no trouble by being clogged. Under no circumstances should undiluted blood be allowed to remain in the pipettes, as it will clot and be very difficult to remove.

IV. Counting the Leucocytes.

The Thoma-Zeiss counter is used after diluting the blood, 1 : 20, in the "white counter" or large pipette. The *best mixing fluid* is a one-half-per-cent. solution of glacial acetic acid in distilled water, which renders the red corpuscles invisible or very shadowy and shows the white corpuscles sharply defined and makes their counting easy. If desired a trace of methyl-violet may be added to the solution, as recommended by Thoma, by which the leucocytes are stained a violet color. The acetic acid solution should be filtered before using, as it is apt to be contaminated with yeasts and fungi. In purchasing the Thoma-Zeiss hæmocytometer the student is advised to ask for the counting slide or grating to be ruled with nine large squares, instead of one, each one millimeter square, which enables the observer to count several fields without the necessity of changing the preparation. Counters, so ruled, are made to order by Zeiss and by Leitz in Germany at a trifling extra cost. The extra rulings will save the observer much time and trouble.

The white corpuscles may be counted, as already stated, with the red corpuscles in the same preparation after diluting the blood in the "red counter" or small pipette with one of the mixing fluids above given, but this method is slow and tedious, requiring several changes of the preparation on the grating in order to obtain a sufficiently large number of white corpuscles for averaging the counts and obtaining accurate results.

V. The Dried and Stained Blood Smear.

The preparation is made as follows:

(*a*) **Making the Smear.**—Obtain a small drop of fresh blood upon a clean cover-glass, reverse this immediately upon a second cover-glass, allowing the blood to spread out in a thin even film between the two glasses, then, quickly and before drying has begun, draw them apart, being careful to move the glasses sideways in their own plane and not to lift them apart; allow both glasses to lie a few minutes with the blood upward until it is dry. These maneuvers must be executed rapidly and require skill to obtain good specimens. The requirements for good smears are that they be thin and evenly distributed. The student is cautioned to protect these preparations from flies, which are attracted to the blood and destroy the specimens in a few minutes.

(*b*) **Fixing.**—The blood must next be fixed upon the glass, for which the most useful methods are the following: (1) *Heat.*—The best results are obtained by heating gradually in a thermostat or hot-air sterilizer to 125° C. and then, before removing, allowing to cool, the process requiring about fifteen or twenty minutes. Good results may also be obtained with experience by passing the slips, held between the fingers, through a Bunsen or alcohol flame about twenty times in rapid succession. This method requires no expensive apparatus and is quick and handy, but uniform success is not obtained with it. If the specimen is to be stained with the Ehrlich mixture for differential counting of the leucocytes, the heat method of fixing is superior to all others. (2) *Absolute alcohol or absolute alcohol and ether.*—The specimen is left in absolute alcohol or in equal parts of absolute alcohol and ether for half an hour or longer and then allowed to dry in the air. This method is adapted to staining the malarial parasites, but is much inferior to the heat method for staining with Ehrlich's triple stain. (3) *Formalin.*—Immerse the specimen for a minute or two in a freshly prepared 0.25-per-cent. solution of commercial formalin (formalin = 40 per cent. formaldehyde) in 95-per-cent. alcohol. This method is the most satisfactory for fixing the malarial parasites (Lazear), but it is not so good as the heat method for fixing the leucocytes for staining with the Ehrlich mixture. The blood may also be fixed by exposing the smear to the vapor of commercial formalin for a few minutes. (4) *Chromic acid.*—*Solley* has recently described a rapid method of fixing blood for staining with Ehrlich's mixture, which he claims gives good results with the differential staining of the leucocytes. By this method the blood film is treated with a two-per-cent. aqueous solution of chromic acid for thirty seconds, washed in running water for a few seconds, dried and stained.

(*c*) **Staining.**—After fixing, the blood-smear is ready to be stained. For general purposes of study and especially for studying the leucocytes and counting them differentially the stain *par excellence* is the Ehrlich triple stain.

EHRLICH'S TRIPLE STAIN.

Orange G, saturated aqueous solution,	6.0
Acid fuchsin, " " "	4.0
Add to these, stirring, drop by drop:	
Methyl green, saturated aqueous solution,	6.6
To this mixture add:	
Glycerin (neutral),	5.0
Absolute alcohol,	10.0
Distilled water,	15.0

REFERENCE:

Solley. Presbyterian Hospital Reports, New York, 1900, Vol. IV., p. 169.

Shake the mixture thoroughly, do not filter, and let stand for a few days before using. Grübler's colors should be used in preparing the mixture. The mixture should be prepared in quantity as it improves with age, though the fresh mixture gives good results.

With this mixture the fixed blood smear is stained for two minutes or more, without heating. It is to be noted that the blood cannot be overstained with this fluid. After staining, wash off the excess of stain in running water for a few seconds and dry between filter paper.

With the Ehrlich mixture the red corpuscles are colored orange, the nuclei of the leucocytes greenish blue, varying in shade and intensity in the different types of leucocytes, the nuclei of erythroblasts almost black, sometimes with a greenish cast, the acidophilic granulation of the polymorphonuclear eosinophiles and eosinophilic myelocytes bright red, the neutrophilic granulation of the polymorphonuclear neutrophiles and neutrophilic myelocytes violet, while the basophilic granulation of the mast-cells remains unstained and stands out clearly as characteristic, small, empty spots surrounding the pale green nucleus. In well-fixed stained slips the color of the slip to the naked eye is orange or orange red, in underheated specimens the color is dark red or reddish brown, and in overheated specimens the color is a pale lemon-yellow.

Much information can be obtained from these stained blood smears. The depth and extent of coloring of the red corpuscles in well-fixed specimens indicates approximately the amount of hæmoglobin, irregularity in size and form (poikilocytosis) is shown, nucleated red corpuscles are seen, the relative proportion of leucocytes is indicated and the classification of the leucocytes into their different varieties is made easy. As already stated, the Ehrlich triple stain is by far the most satisfactory stain for general work, revealing, as it does, so much of the finer structure of the blood, and, as Cabot keenly observes, "To 'control' Ehrlich's triple stain with eosin-hæmatoxylin or eosin-methylene-blue is like controlling a chronometer with a fifty-cent clock."

Jenner's Stain.—This is a very useful stain for bedside use, as it *fixes and stains* at the same time the blood smear. It is made by dissolving 0.5 gram of Jenner's powdered dye in 100 cc. of alcohol, or the fluid dye can be obtained ready for use from the dealers. The ordinary dried blood slip is covered with this stain from one to four or five minutes, washed in water, dried between filter paper, and mounted in xylol-balsam for examination. All the cells are stained differentially, and malarial parasites are also stained. For immediate examination this stain is useful, but it is far inferior to Ehrlich's triple stain as a differential leucocyte stain.

For special purposes other stains are useful, of which a few will be given, as follows:

For Malarial Parasites. [Fixing should be done by absolute alcohol, absolute alcohol and ether, or formalin, not by heat.]

1. Treat the specimen with a 0.5-per-cent. solution of eosin in 60-per-cent. alcohol for one minute, wash in water, dry, apply a saturated aqueous solution of methylene-blue or Delafield's hæmatoxylin for five minutes or longer, wash in running water, and dry between filter paper.

2. *Chenzinsky-Plehn Solution.* [Fix for five minutes in absolute alcohol.]

Saturated aqueous solution of methylene-blue,	40.0
0.5-per-cent. eosin solution in 70-per-cent. alcohol,	20.0
Distilled water,	40.0

Filter before using, and stain the slips from six to twenty-four hours, best twenty-four hours, wash and dry.

3. *Futcher and Lazear's Solution.* [Fix in formalin, wash, and dry.]

Saturated solution of thionin in 50-per-cent. alcohol,	20.0
2-per-cent. solution (aqueous) of carbolic acid,	100.0

Preserve for several months before using. Stain the slip for twenty seconds, wash and dry.

The above three stains are useful and sufficient for staining the malarial parasites for diagnostic purposes, but for differentiating the finer structure of the parasites, and especially their nuclear chromatin, the Romanowsky stain or one of its modifications is required.

4. *Romanowsky's Stain:*

To one part of an old saturated aqueous solution of methylene-blue add, gradually, stirring, *about* two parts of an aqueous 1-per-cent. eosin solution until the commencing formation of an insoluble precipitate imparts a violet tint to the mixture. Without filtering, carefully remove from the surface a violet scum with metallic luster which forms upon it. Upon the surface of this fluid, always freshly made, float the reversed blood slips, cover the dish to prevent evaporation, and let stand for one to three hours, according to the intensity of staining desired. Wash the stained slips thoroughly in distilled water and dry. The exact proportion of the two staining solutions required to produce the violet tint in the mixture varies slightly according to the special manufacture of the colors. Gautier advises the use of only particular brands of methylene-blue and eosin made by the Badische-Soda-Anilin-Fabrik. Ziemann prefers the Methylenblau medic. puriss. (Höchst), the Methylenblau rectificat. of Grübler, and eosin A.G. and B.A. (Höchst), and modifies the proportions to 1 : 4 to 1 : 7 of a 1-per-cent. aqueous solution of methylene-blue and a 0.1-per-cent. aqueous solution of eosin, respectively, staining with this mixture from twenty to forty minutes. The blood should be fixed in absolute alcohol for half an hour before using the Romanowsky stain.

Nocht discovered that the unknown nucleus-staining substance of this mixture was present in polychrome-methylene-blue, from whatever factory it was obtained. Lazear recommends the following technique, as a modification of Romanowsky's method, for giving the most exquisite results:

Two solutions are prepared as follows: (α) Dilute acetic acid is added to polychrome-methylene-blue until it is feebly acid as shown by litmus paper. When litmus paper is dipped in the solution it is colored by the methylene-blue, but at the margin of the moist portion of the paper a red line caused by the acid can be seen. More of the polychrome-methylene-blue is now added until the solution is neutral in reaction. To the neutralized polychrome-methylene-blue solution an equal volume of water is added and then enough saturated aqueous solution of methylene-blue to cause the polychrome color of the solution to disappear. (β) A 0.2-per-cent. aqueous solution of eosin. Place these two solutions in burettes, and to 4 cc. of β add α drop by drop until the red color has just completely disappeared. A few experiments will show the exact proportion to use to obtain the best results, and thereafter the exact amounts can be easily measured from the burettes. The two solutions should be mixed just before use in a covered staining dish. The specimen should be left in the stain for from three to twenty-four hours. A scum which forms on the surface of the stain should be removed carefully with a piece of filter paper just before placing the specimen in it and again just before taking it out.

"The red corpuscles take a dark-red color and the nuclei of the leucocytes

REFERENCE:

Lazear. Pathology of Malarial Fevers, Structure of the Parasites and Changes in the Tissue. Journ. Amer. Med. Assoc., Oct. 13, 1900, p. 917.

and of the nucleated red corpuscles take a deep carmine-violet. The protoplasm of the malarial parasite stains blue. The nucleus of the malarial parasite is seen to be made up of a peripheral unstained or achromatic portion and a central mass or cluster stained a deep carmine-violet, which is chromatin and is sometimes spoken of as the nucleolus."

For Basophilic Granules.—The basophilic granules of the mast-cells and of certain red corpuscles ("*granular degeneration*") are stained by any basic dye. The basophilic granules of the mast-cells have the peculiarity of not taking the pure color of most stains, but of taking "off-colors" (metachromatism). These granules are well stained by a saturated aqueous solution of methylene-blue or better by the following mixture:

Dahlia, saturated solution in absolute alcohol,	50.0
Distilled water,	100.0

Upon clearing add 10–12.5 parts of glacial acetic acid. Stain the specimens with this solution for from five minutes to twenty-four hours, wash in water and dry.

The following modification of Ehrlich's triple stain is used by Neusser to stain (blue) the "*perinuclear basophilic granules*":

Acid fuchsin, saturated aqueous solution,	50.0
Orange G., saturated aqueous solution,	70.0
Methyl green, saturated aqueous solution,	80.0
Distilled water,	150.0
Absolute alcohol,	80.0
Glycerin,	20.0

For Diabetic Blood.—Bremer's Test. [The blood slips are gradually heated to 135° C. and then allowed to gradually cool.]

Stain thickly smeared blood slips for two minutes in a one-per-cent. aqueous solution of Congo red, wash and dry. Diabetic blood looks *yellow* to the naked eye, whereas normal blood so stained looks *red*. Methyl blue may be used instead of Congo red, giving in diabetic blood a yellowish green color (in normal blood a blue color).

Williamson's Modification of Bremer's Test.

Make the following mixture:

Blood,	0.02
Aqueous solution of methyl blue, 1 : 6000,	1.00
Potassium hydrate solution, 60 per cent. (sp. gr. 1.058),	40.00
Distilled water,	40.00

Allow this mixture to stand in boiling water for three or four minutes. A yellow color of this mixture is characteristic of diabetic blood.

This reaction is claimed by Bremer to occur only in diabetes, whether frank with glycosuria, or latent without showing sugar in the urine. The reaction has been found by other observers occasionally in Graves' disease, leukæmia, Hodgkin's disease, and multiple neuritis.

For Glycogen Granules.—Glycogen granules, whether free in the plasma or contained within the leucocytes, are shown by the iodine reaction. The blood dried at ordinary temperature is treated for a few minutes with the following solution:

Iodin sublim.,	0.5
Potass. iodid.,	1.5
Aq. dest.,	50.0
Muc. acaciæ ad consist. syruposam.	

Mount in this solution and examine fresh. The glycogen granules are stained mahogany-brown.

A still better method of staining is the following: Place the preparations in a covered dish containing some crystals of iodine and allow the fumes of the iodine to act for a few minutes. Mount the slips in a saturated solution of levulose of high refractive index. The granules of glycogen are stained as by the iodine-gum solution. For preserving the amounts, the cover-glass must be sealed with cement (Ehrlich and Lazarus).

(*d*) **Mounting.**—After the blood smears have been fixed, stained and washed, as described, they may be mounted on a slide in the usual way in Canada balsam, when they are ready to be examined with the oil immersion lens.

VI. Determining the Coagulation Time.

The blood is sucked up into several coarse capillary tubes (Wright's "coagulometers"). These are tested at intervals of one minute by blowing out the blood to determine the length of time required for clotting within the tube, normally from three to five minutes.

VII. Bacteriological Cultures.

Cultures from the blood are made with value in certain infections, pyæmia, etc., in order to determine the microörganism invading the blood. Due to the normal presence of the Staphylococcus epidermidis albus (Welch) deep in the hair follicles and sebaceous and sweat glands of the skin, so that thorough disinfection of the skin is impossible, it is best not only to sterilize the skin so far as possible but also to incise the skin and expose a blood vessel, preferably a vein, which is punctured by the sterile aspirating needle in order to withdraw blood for bacteriological examination and culture. Because of the germicidal action of dead blood, it is advisable, as recently shown by Adami, to place the blood withdrawn in a large amount of culture medium, which by largely diluting the blood reduces and opposes its germicidal action. As only few bacteria may be present in the circulating blood, several cubic centimeters of blood should be withdrawn and then placed in from a half liter to a liter of the fluid culture medium. The usual test-tube cultures are not reliable and should be replaced by the newer method here described. The further examination for bacteria in the culture medium should be conducted in the usual way.

VIII. The Widal Agglutination Reaction.

This subject is too technical and extensive to be considered in this limited chapter. The student is referred for practical information to Cabot's "Serum Diagnosis."

General Considerations of the Physiology and Pathology of the Blood.

The Red Corpuscles.—The red corpuscles are increased or diminished in the peripheral circulation in many physiological and pathological states, as follows:

Increased in concentration of the blood from stasis and cyanosis whether local, from pressure, or general, as in chronic heart disease; in increased local blood pressure due to vasomotor constriction from emotions, cold, severe exercise, massage, electricity, etc.; in high altitudes above sea-level; after a drain upon the watery constituent of the blood, as from profuse diarrhœa, vomiting, sweating, diuresis, and immediately following a rapidly accumulating large serous effusion; from starvation of liquids; in general, in lean persons, and in the first week of life after birth, etc.

Diminished.—After rapid absorption into the circulation of large quantities of fluid (drinking, hypodermoclysis); with lowered blood pressure, as in acute loss of compensation in heart disease, and due to peripheral vasomotor dilatation, as from emotions, hot baths, drugs, etc.; after parturition and hemorrhages, and during lactation, puberty, senility, and, in general, in females (normal), and from all causes producing anæmia.

The various factors affecting the count of the red corpuscles must all be carefully considered and a balance reached in the judgment before drawing conclusions in any given case under examination. A careful consideration of the size, form and color of the red cells and of the presence or absence of nucleated red corpuscles, polychromatophilia, "granular degeneration," etc., will aid materially in judging of the number and condition of the red corpuscles.

Poikilocytosis, polychromatophilia, and *granular degeneration* are characteristic of severe anæmias.

Nucleated red corpuscles are found usually in severe anæmic states, and in conditions of debility in young children whose blood tends to revert partially to the fœtal type. Normoblasts are the forms usually seen. Megaloblasts in abundance are found chiefly in pernicious anæmia. Mitosis of the nuclei is rarely seen.

Rouleaux formation should be looked for in the fresh blood, as its absence is pathological and a sign of severe anæmia.

Hæmoglobin.—The hæmoglobin is increased in conditions of concentration of the blood, and is diminished in states of dilution of the blood and in anæmia. It is to be noted that the hæmoglobin is not usually reduced proportionally to the red cells in anæmia. By dividing the percentage (of the normal amount) of the hæmoglobin by the percentage (of the normal number) of the red corpuscles, the "*color-index*" of the individual corpuscles is obtained, being minus or plus according as the hæmoglobin content is below or above the normal standard of 1. In secondary anæmias and in chlorosis the "color-index" is minus, and in primary pernicious anæmia it is usually plus, in the latter case due to the increased average size of the red corpuscles.

The Anæmias.—The anæmias are usually classified as (*a*) primary, *i. e.*, idiopathic and *without apparent cause*, and (*b*) secondary, *i. e.*, dependent upon known or supposed antecedent factors. The primary anæmias include only pernicious anæmia and chlorosis, while the secondary anæmias include all other anæmic conditions. This division of the anæmias as primary and secondary is unscientific and at best but a temporary convenience, for it is inconceivable that the "primary" anæmias (pernicious anæmia and chlorosis) are without antecedent causative factors. However, for the present, it may be as well to allow the time-honored classification to stand.

The general characteristics of *secondary anæmias* are as follows: The red corpuscles and the hæmoglobin are both reduced, the latter in greater proportion than the former, so that the color-index is minus. The leucocytes may or may not be increased or diminished, depending upon the cause of the anæmia. Nucleated red corpuscles are usually present in severe secondary anæmias, chiefly normoblasts, and poikilocytosis, polychromatophilia, and granular degeneration may also be seen.

The *primary anæmias* show no characteristic changes in common. Both chlorosis and pernicious anæmia present individual pictures. *Chlorosis* resembles the general type of secondary anæmias, *i. e.*, with a greater relative reduction of hæmoglobin than of the red corpuscles, but it shows a marked characteristic in the fact that the color-index is extremely low (average, 0.4), due to a slight reduction of red corpuscles and a very great reduction of hæmoglobin.

Pernicious anæmia is characterized by a maximal reduction of both the red corpuscles and the hæmoglobin, the former usually in greater degree than the latter, so that the color-index is plus in typical cases, though not invariably so. Nucleated red corpuscles are very abundant, of which the megaloblasts predominate over the normoblasts. Great poikilocytosis is the rule and the average size of the red corpuscles is increased above the normal (macrocytosis).

The Blood Platelets.—The platelets are said to be increased in some diseases, notably chlorosis and leukæmia, and diminished in other conditions, hæmophilia, purpura, malaria, etc. The significance of their number is unknown and at present little practical information can be obtained from their study.

The Leucocytes.—The leucocytes are characteristically increased or diminished in certain conditions and their absolute and differential counting are of great practical value in clinical medicine, often being decisive in making a diagnosis.

An increase of leucocytes above 10,000 per cubic millimeter (leucocytosis) is found in certain physiological and pathological processes, as follows :

Physiological Leucocytoses.—The physiological leucocytoses occur in the following conditions :

1. **Infancy.**—The leucocytosis may reach as high as 30,000 per cubic millimeter just after birth, gradually falling to about 12,000 by the sixth month and to below 10,000 after the first few years of life. The differential leucocyte count in infant's blood shows marked differences from the count in adults. The lymphocytes are normally 40–60 per cent., the small variety predominating ; the neutrophiles are 30–50 per cent., and the eosinophiles 3–8 per cent. Myelocytes are not uncommon. Young children show a strong tendency to revert to the type of blood of infancy in any condition of debility or retarded development, as in rickets.

2. **Pregnancy.**—A moderate leucocytosis is found in advanced pregnancy in primiparæ, but occurs in only a portion of cases in multiparæ. All the varieties of the leucocytes are increased proportionally.

3. **Post-partum.**—A distinct leucocytosis is the rule, being highest immediately after delivery and gradually disappearing in the course of a few weeks. This leucocytosis cannot be ascribed to puerperal infection, as it occurs in non-septic cases.

4. **Digestion.**—After a meal, especially if abundant in proteids, leucocytosis normally occurs. It is apparent at the end of an hour and increases for about three or four hours. It is most marked in strong vigorous persons and in infants. The increase usually measures about thirty-three per cent. (Rieder). Digestion leucocytosis may be absent in conditions of debility, gastro-intestinal disturbances, and in a majority of cases of carcinoma of the stomach (in 34 out of 37 cases examined at the Massachusetts General Hospital).[1] The increase in the leucocytes was found by Burian and Schur to be chiefly in the neutrophiles, but most observers have reported little change in the relative proportion of the different varieties of leucocytes, some finding a slight lymphocytosis. In the fasting state the leucocyte count is lowered.

5. **Before Death.**—"Terminal," "agonal," or "moribund" leucocytosis frequently occurs just before death. It is believed by Ehrlich and Lazarus to be only apparent and due to blood stasis. In pernicious anæmia the leucocytes may rarely rise before death so high as to simulate leukæmia (Cabot).

6. **Violent Exercise, Massage, Cold Baths and Electricity.**—All these factors tend to increase the leucocytes, probably by vasomotor constriction. Prolonged cold baths leading to vasomotor paralysis lower the count. The different kinds of leucocytes are increased proportionally.

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[1] Few observers confirm the very high rate here shown.

Pathological Leucocytoses.—A pathological increase of leucocytes occurs as follows:

1. **Inflammatory Leucocytosis.**—In most inflammatory processes a distinct and characteristic leucocytosis occurs. The differential count shows the neutrophiles relatively increased, as in fact they are absolutely. Inflammatory leucocytosis is not necessarily dependent upon the formation of pus, for it occurs in non-purulent as well as purulent processes, *e. g.*, erysipelas, empyema, etc.

The degree of leucocytosis depends upon two factors, (*a*) the severity or virulence of the infection and (*b*) the vitality or reacting power of the individual. A mild infection tends to produce only slight leucocytosis or none at all, whereas a severe infection causes marked leucocytosis. On the other hand, given an inflammatory process, the greater the vitality and reacting power of the individual the higher the leucocytosis tends to become, within certain limits. Marked loss of vital reaction may, even in severe infections, prevent the production of leucocytosis. The toxicity of acute virulent septicæmia may be so overwhelming as to crush the reacting power and prevent the increase of the leucocytes. The interdependence of the two factors is shown in the following table, from Cabot:

Infection mild: vital reaction good = small leucocytosis.
Infection less mild: vital reaction less good = moderate leucocytosis.
Infection severe: vital reaction good = very marked leucocytosis.
Infection severe: vital reaction poor = no leucocytosis.

The absence of leucocytosis in an inflammatory disease, showing otherwise symptoms of great severity, is of grave omen and a prognosis of death is sometimes possible from this consideration. The absence of leucocytosis, therefore, in an inflammatory affection, in which leucocytosis is as a rule present, may be taken usually as a sign either of a very mild infection or of a virulent infection with low vital reaction. These principles are well exemplified in pneumonia.

Before inflammatory leucocytosis develops there is sometimes observed a transient leucopenia (polymorphonuclear), as though the toxin produced a shock on the leucocytes before they accommodated themselves to meet the assault by marshaling their forces in increased array (Löwit). There is reason, however, to think that this temporary leucopenia is only apparent and is due to an altered distribution of the leucocytes by which they are increased in the deeper circulation at the expense of the peripheral circulation (Goldscheider and Jakob).

A rapidly extending inflammation shows, as a rule, a greater leucocytosis than a chronic or stationary process shows. A spreading infection tends also to cause an *increasing* leucocytosis, whereas a stationary process, as in a walled-off appendicular abscess, shows a stationary leucocyte count. An unusually high leucocytosis is a bad sign, indicating a very severe infection. To summarize, in an inflammatory affection such as is normally accompanied by leucocytosis, the absence of leucocytosis is either a serious omen, showing virulent infection with slight reacting power, or a sign of unusually mild infection, while an exceptionally high leucocytosis is a sign of great severity of infection (with high reacting power) and makes the prognosis grave.

2. **Toxic and Drug Leucocytosis.**—This division includes a heterogeneous group of agents that are thus classed for want of a better explanation of their usual or occasional production of leucocytosis. A few only may be mentioned, as ether and chloroform (during and following anæsthesia), nuclein, hypodermoclysis of normal saline solution, illuminating gas poisoning, certain ptomaines, potassium chlorate, arsenic, phenacetin, the derivatives of phenyl-hydrazin, camphor, etc. The action of drugs is exceedingly erratic in producing leucocy-

tosis, as a rule large and sometimes toxic doses being required to produce an increase of the white cells.

3. **Post-hemorrhagic Leucocytosis.**—Shortly after the occurrence of a *large* hemorrhage a well-marked leucocytosis may appear, consisting usually of an increase of the polymorphonuclear neutrophiles. This leucocytosis is transient and soon disappears.

4. **The Leucocytosis of Malignant Tumor.**—The leucocytes may be increased in both carcinoma and sarcoma.

(*a*) **Carcinoma.**—A leucocytosis is the rule in carcinoma, many exceptions, however, occurring. The increase consists usually of the polymorphonuclear neutrophiles, with a tendency toward the occurrence of occasional myelocytes. In general, the more malignant, rapidly growing and extensive the growth the greater is the leucocytosis. In slowly growing scirrhus, cancer of the lip, etc., with little constitutional disturbance and no cachexia, the leucocyte count is usually normal or only slightly increased. The vital resistance of the individual, also, probably plays a part in determining the reaction of leucocytosis. The location of the growth is also important with reference to the facility of absorption of the toxins assumed to be elaborated by the malignant growth. The finding of a leucocytosis, not otherwise accounted for, in a case of suspected carcinoma, is of course of greater diagnostic significance than the absence of leucocytosis would be.

(*b*) **Sarcoma.**—In general, the leucocytosis in sarcoma is more marked and more constant than in carcinoma.

5. **The Leucocytosis of Leukæmia.**—See Leukæmia below.

Leucopenia.—A subnormal leucocyte count is found in certain conditions, as in malnutrition, starvation, after short hot baths and prolonged cold baths, in pernicious anæmia, and often in the course of such infectious diseases as do not normally show leucocytosis, viz., typhoid fever, malaria, epidemic influenza, tuberculosis (pure), etc. Leucopenia is apt to be characterized by a relative increase of the lymphocytes and decrease of the neutrophiles.

The Differential Leucocyte Count.—Counting the leucocytes differentially and finding the relative number of each variety is of practical value, as the proportion of neutrophiles, eosinophiles, small and large lymphocytes and myelocytes varies in different morbid processes. The leucocytosis of leukæmia is differentiated from that of inflammatory diseases, lymphatic leukæmia is distinguished from spleno-myelogenous leukæmia, the diagnosis of trichinosis is made easy and the nature of many morbid processes is illuminated by the differential leucocyte count.

Glycogen is sometimes found in the polymorphonuclear neutrophiles, rarely in the plasma, either diffuse or in the form of granules of varying size, and is demonstrated by the iodine reaction. Czerny's investigations, however, throw doubt on the identity with glycogen of this iodine-staining substance, which partakes of the characteristics of both glycogen and amyloid and may perhaps in individual instances be either or an intermediate product standing between the two. It is most commonly found in conditions of severe inflammation and sepsis, associated with leucocytosis, and is of some value in the differential diagnosis of such conditions in obscure cases (Goldberger and Weiss). It may be present in the blood in diabetes, and occasionally in small amount in other conditions.

Neusser's perinuclear basophilic granules are without known significance. They were claimed by Neusser to be diagnostic of "uric-acid diathesis," but the researches of Futcher, Simon, and others have disproved this, as such granules are found in varying proportions in normal blood. They are especially frequent in the blood of gout and leukæmia. Ehrlich and Lazarus believe that they are the products of the use of impure dyes.

Fibrin.—The fibrin network formed in the fresh blood slip after standing about fifteen minutes or longer is diminished or increased in certain conditions, and its approximate estimation by observing the closeness of the network is of some diagnostic value. Increased fibrin formation accompanies the leucocytosis of inflammatory disease. In malignant growths the fibrin network of the blood remains normal or may become subnormal. The estimation of the fibrin is therefore of some importance in assisting to distinguish between the leucocytosis of inflammatory disease and malignant tumor. The greatest increase of fibrin is seen in pneumonia, acute articular rheumatism, and purulent processes. In pernicious anæmia the fibrin formation is subnormal, and it is not increased in typhoid fever, malaria, post-hemorrhagic anæmia, chlorosis, hysteria, leukæmia, etc.

The Coagulation Time.—In certain pathological states, *e. g.*, purpura, hæmophilia, chronic obstructive jaundice, etc., the coagulation time is retarded, in some cases even to as much as an hour. The determination of the coagulation time is of especial importance in surgical cases, in which retarded coagulation may be of serious moment.

Special Pathology.

The application of the above facts and principles to special diseases may be here undertaken only with the greatest brevity and in a limited number of conditions in which blood examination is of special clinical value. The different affections and organs will be taken up, as far as possible, in alphabetical order.

Abscess.—Red corpuscles and hæmoglobin—no change in acute cases, changes of secondary anæmia in chronic cases. Leucocytosis is the general rule, with increased neutrophiles (neutrophilia). Glycogen granules may occur in the leucocytes. Fibrin increased. (See Inflammatory Leucocytosis.) *"Cold" abscess* is without leucocytosis or fibrin increase.

Addison's Disease.—No changes in the early stage; in advanced cases, secondary anæmia without leucocytosis. Melanæmia has been exceptionally observed.

Adenitis.—*Acute suppurative adenitis* shows inflammatory leucocytosis and increased fibrin formation. *Chronic tubercular adenitis* may show secondary anæmia, and is without leucocytosis unless secondary septic infection of the glands occurs. *Syphilitic adenitis* may show slight leucocytosis. *Glandular metastases from carcinoma and sarcoma* show leucocytosis without increased fibrin. *Hodgkin's disease* is without leucocytosis. *Lymphatic leukæmia* shows the characteristic blood picture of that disease.

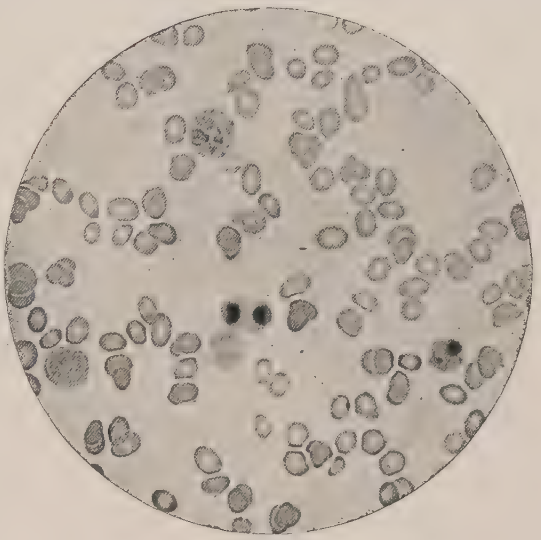
Anæmia Infantum Pseudoleukæmica (von Jaksch).—Von Jaksch described under this name a condition of great splenic enlargement associated with grave secondary anæmia and marked leucocytosis *in infants*. Later investigations have made it probable that this condition is merely one of severe secondary anæmia with enlarged spleen due to rickets or syphilis. (See Fig. 45.)

Anæmia, Primary.—See Chlorosis and Pernicious Anæmia.

Anæmia, Secondary.—Reds reduced; hæmoglobin reduced in greater proportion than the reds; in advanced cases poikilocytosis, polychromatophilia and granular degeneration occur, occasional nucleated reds, usually normoblasts, may be seen and rouleaux formation may be absent. The number of the leucocytes depends upon the nature of the morbid process causing the anæmia, according to the principles above set forth. (See Plate XXXVI., Fig. 3.)

Fibrin is increased when inflammatory disease is the cause of the anæmia. In *young children* most causes leading to anæmia produce more marked changes in the blood than in adults. Their blood tends to revert toward the fœtal type. Degenerative changes are pronounced and nucleated reds may be abundant, especially megaloblasts. The leucocytes are usually much increased and lymphocytosis is strongly marked. Such changes in infants' and young children's blood have far less significance than they have in adults' blood. **Differential diagnosis**: From *chlorosis*, by the age, sex, and history of the patient, and in general by the lower color-index in chlorosis; uncomplicated chlorosis is without leucocytosis or increased fibrin, whereas secondary anæmia is often due to causes producing them and at the same time neutrophilia. Some severe cases may be indistinguishable from chlorosis by blood examination. In this connection it must be remembered that chlorosis is probably only a special type of secondary anæmia, although classed under the primary anæmias. From *pernicious anæmia*, by the high color-index of the latter, by the great oligocythæmia, the greater average size of the reds, the great frequence of nucleated reds (of which the megaloblasts usually predominate), and the greater degenerative changes of the red corpuscles in pernicious anæmia. Leucopenia and lymphocytosis are the rule in pernicious anæmia, but may occur in secondary anæmias, as in malaria, typhoid, etc.

FIG. 45.



Anæmia Infantum Pseudoleukæmica. (von JAKSCH.)

Anæmia Splenica.—This disease, characterized by primary and progressive enlargement of the spleen, progressive anæmia without leucocytosis, and a tendency to hæmorrhage, œdema, and ascites in the advanced stages, ending in death, has been described under various names, *e. g.*, *anæmia splenica*, *pseudoleukæmia splenica*, *splenomegalie primitive*, *Banti's disease*, the *splenic form of Hodgkin's disease*, etc. These different names are all applied, without apparent distinction,[1] to the symptom-complex above outlined. The blood picture is that of a progressive secondary anæmia without leucocytosis and usually with marked leucopenia. No characteristic changes in the differential leucocyte count have been found. The average red count in fourteen of Osler's cases was 3,336,357 and the hæmoglobin was relatively low, while of thirteen cases in which the leucocytes were counted, nine showed a count below 5,000, six of which had extreme leucopenia, and one case only (Case VI.) showed a slight leucocytosis. Poikilocytosis and nucleated reds, both normoblasts and megaloblasts (see Osler's Case XIII.), occur in severe cases.

So few cases of this disease have been carefully studied in respect to the blood that it may be well to give space here to recording the blood examinations of three cases (unreported) that have fallen under the writer's personal observation, in Buffalo, during the past two years.

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[1] Banti, however, describes slight atrophic cirrhotic changes in the liver in advanced cases, secondary to a supposed elaboration of toxin in the spleen. Such a pathological change seems hardly sufficient to separate Banti's disease from other forms of splenic anæmia.

Case I. Mr. G. S., aged 59.

February 21, 1899.

Hæmoglobin,	49 per cent.	Neutrophiles,	81.6 per cent.
Reds,	2,982,400	Small lymphocytes,	6.0 “
Whites,	4,800	Large “	5.6 “
		Myelocytes,	6.8 “
		Eosinophiles,	0.0 “

Marked poikilocytosis and 28 normoblasts in counting 1,000 leucocytes; no increase of fibrin.

May 15, 1899.

Hæmoglobin,	41 per cent.	Neutrophiles,	69.8 per cent.
Reds,	1,776,000	Small lymphocytes,	8.8 “
Whites,	3,075	Large “	4.8 “
		Eosinophiles,	2.6 “
		Neutrophilic myelocytes,	13.8 “
		Eosinophilic “	0.2 “

Marked poikilocytosis; frequent normoblasts and occasional megaloblasts; fibrin normal.

A previous examination (Dec. 29, 1898) made by Dr. A. E. Woehnert, of Buffalo, showed:

Hæmoglobin,	45 per cent.	Neutrophiles,	72 per cent.
Reds,	2,685,000	Small lymphocytes,	23 “
Whites,	4,000	Large “	3 “
		Eosinophiles,	2 “
		Mast cells,	occasional.
		Myelocytes,	“

Marked poikilocytosis, megaloblasts present, fibrin normal.

This case died May 29, 1899. Autopsy was made by the writer, confirming the diagnosis. The spleen weighed 1,270 grams, the liver 2,280 grams. The progressive increase of myelocytes is especially interesting.

Case II. Mrs. X., aged about 45.

May 28, 1899.

Hæmoglobin, not estimated.		Neutrophiles,	70 per cent.
Reds, not counted.		Small lymphocytes,	19 “
Whites,	3,300	Large,	10 “
		Eosinophiles,	1 “

Reds well formed, somewhat pale; no nucleated reds seen; fibrin normal.

This case is still (June, 1901) living. The spleen is of very large size.

Case III. Mr. V. B., aged 40.

May 15, 1900.

Hæmoglobin,	61 per cent.	Neutrophiles,	76 per cent.
Reds,	4,160,000	Small lymphocytes,	13 “
Whites,	3,120	Large “	9 “
		Eosinophiles,	2 “

Reds showed considerable variation in size, but no deformity; no nucleated reds; fibrin normal.

The history, course, and physical examination were all characteristic. The case is still (June, 1901) alive.

Differential Diagnosis.—From *pernicious anæmia*, which is sometimes associated with considerable splenic enlargement, great difficulty may be experienced in certain cases. The lower grade of splenic enlargement in pernicious anæmia, the high color-index, and the constant occurrence of erythroblasts in large numbers, a majority of which are megaloblasts, as well as the clinical history, all serve to differentiate it from splenic anæmia. From *malignant tumor of the kidney*, by the leucocytosis of the latter. From *cirrhosis of the liver with splenic enlargement*, the distinction may be very difficult and tax the resources of the best clinician. The history of alcoholism, syphilis, etc., and the previous course of the disease may assist. In syphilitic cases a leucocytosis is sometimes found. In general the distinction is easy, as the splenic enlargement in cirrhosis of the liver is usually only moderate and the anæmia is less marked. From *splenic leukæmia*, by the characteristic blood condition of the latter. From *ague-cake spleen*, by finding malarial parasites in the blood, or by the history of chronic malaria, etc.

Apoplexy.—See Brain.

Appendicitis.—*Catarrhal appendicitis* is only occasionally accompanied by leucocytosis. If the process has gone beyond the catarrhal stage, typical inflammatory leucocytosis with increased fibrin formation occurs. The only exceptions are the mildest and severest cases. This disease follows typically the general laws governing inflammatory leucocytosis described under Physiology and Pathology above. **Differential diagnosis**: From *typhoid fever* (without inflammatory complications) by the absence of leucocytosis and of fibrin increase and by the presence of the Widal reaction in the latter. From *fecal impaction* by the absence of leucocytosis in the latter. From simple *renal*, *intestinal*, *uterine*, and *biliary colic*, when not associated with inflammatory changes, by the absence of leucocytosis in the latter. From *floating kidney, hydronephrosis, and simple cysts*, by the same point. From other *abscesses* in the pelvic region, the blood examination does not suffice to differentiate.

Arthritis.—*Acute rheumatic, septic, and gonorrheal arthritis* show the blood changes of inflammatory diseases.

Asthma, Bronchial.—A well-marked eosinophilia usually characterizes the period of and just after the paroxysm. Concentration of the blood may also occur during the paroxysm. **Differential diagnosis**: From *cardiac* and *renal asthma*, by the absence of eosinophilia in the latter.

Brain Diseases.—*Abscess* shows inflammatory leucocytosis.

Apoplexy (hemorrhage) is usually associated with leucocytosis.

Meningitis of all kinds, excepting tuberculous, shows well-marked leucocytosis. Tuberculous meningitis also is usually associated with leucocytosis, but exceptions are common. **Differential diagnosis**: Given a meningitis, the absence of leucocytosis speaks for its tuberculous nature. Typhoid fever with cerebral manifestations is distinguished from meningitis (except tuberculous) by the absence of leucocytosis in the former. From brain tumor and hysteria, a leucocytosis usually distinguishes meningitis.

Syphilis of the brain is said to show leucocytosis.

Thrombosis of the sinuses and veins in a case of chlorosis, observed by the writer, showed a well-marked leucocytosis. Capillary hemorrhages in the basal

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Osler. On Splenic Anæmia. Amer. Journ. Med. Sci., January, 1900.

ganglia may, however, have accounted for the leucocytosis. Negative bacteriological examination and cultures excluded secondary infection in this case.

Tumors of the brain are said not to show leucocytosis.

Bronchitis.—Acute and chronic bronchitis involving the larger tubes usually show no leucocytosis, but may exceptionally do so. Acute capillary bronchitis involving the finer tubules shows leucocytosis. When associated with chronic emphysema an eosinophilia may occur. **Differential diagnosis**: From *pneumonia*, the absence of leucocytosis argues strongly in favor of bronchitis, while the presence of a moderate leucocytosis is not decisive between the two.

Bubonic Plague.—The leucocytes are greatly increased and a clump reaction occurs after the first week.

Carbon Monoxide Poisoning.—Concentration of the blood is seen in most cases. Marked leucocytosis is the rule. The color of the blood, as a whole, is bright cherry red. The spectrum of the blood is characteristic.

Carcinoma.—See Malignant Tumor.

Cerebral Diseases.—See Brain.

Cerebro-Spinal Meningitis (Epidemic).—A marked leucocytosis is the almost invariable rule.

Chicken Pox.—A leucocytosis was observed by Engel during the pustular stage.

Children.—The blood picture in infants and young children differs markedly from that of adults. (See Infants.)

The general rules governing changes in the blood of adults cannot be strictly applied to young children.

Chlorosis.—The red corpuscles are, as a rule, reduced only moderately, whereas the hæmoglobin is relatively very low, hence *a low color-index* (average 0.40). The average size of the red corpuscles is reduced. Poikilocytosis and degenerative changes are marked only in severe cases. Nucleated red corpuscles are uncommon, and occur chiefly as normoblasts. The leucocytes are normal in number, reduced in severe cases. There is usually a relative lymphocytosis at the expense of the neutrophiles, and there may be a slight increase of eosinophiles. The blood platelets are said by most writers to be greatly increased. Fibrin is not increased. Chlorosis is classed as a primary anæmia, but is better regarded as a special form of secondary anæmia occurring in young women. **Differential Diagnosis**: From *secondary anæmia*, see Anæmia, Secondary. The absence of leucocytosis in uncomplicated chlorosis is an important guide in differential diagnosis. The age and sex of the patient and the low color-index are the important points in the diagnosis.

Cholangitis.—See Gall Bladder and Ducts.

Cholera Asiatica.—Due to the profuse watery evacuations there is concentration of the blood and the red cells are increased. A high leucocytosis is the rule, with neutrophilia. The alkalinity of the blood is greatly diminished in the last stages, and, indeed, an acid reaction may occur.

Cirrhosis.—See Liver.

Colic.—*Biliary, intestinal, uterine and renal colic*, when simple and not accompanied by inflammatory trauma, do not show leucocytosis.

Cystitis.—Usually there is no leucocytosis. Cabot has found leucocytosis in a few severe acute cases.

Cysts.—In simple non-malignant cysts the blood is normal.

Diabetes Mellitus.—The red count varies widely, due to the influence of the sugar in the blood in leading to dilution and concentration by diuresis and increased absorption from the tissues. In advanced stages a well-marked secondary anæmia is found. The leucocytes are said by Cabot to be normal in number, with which the writer's experience agrees. Lipæmia has been found in some cases. Glycogen granules may be found in the plasma and leucocytes. The blood is characterized by Bremer's test. Diabetic coma does not as a rule show leucocytosis.

Diphtheria.—In some cases a slight secondary anæmia develops, though most cases, especially if treated with antitoxin, show the reds and hæmoglobin about normal. Leucocytosis is the rule, with exceptions in the mildest and severest cases (Billings, Ewing, Morse). Antitoxin does not influence the leucocyte count. A relative neutrophilia occurs with the leucocytosis. Engel reports finding myelocytes up to 16.8 per cent. in fatal cases. **Differential diagnosis**: As most cases of follicular tonsillitis show leucocytosis, the blood examination does not distinguish between it and diphtheria. **Prognosis**: The absence of leucocytosis in manifestly severe cases shows poor vital reaction and is a bad sign.

Dysentery.—See Intestine.

Echinococcus Cysts.—Few observations are recorded. Hayem found leucocytosis and increased fibrin in hydatid cyst of the liver. The writer found the leucocyte count and fibrin formation normal in a single case of primary exogenous echinococcus cysts of the pleura, without inflammatory degeneration.

Emphysema.—Concentration may be present and an eosinophilia has been noted in some cases. Further observations are required.

Empyema.—Purulent pleurisy shows the typical blood picture of abscess. (See Abscess.) **Differential diagnosis**: Serous pleural effusions, whether tuberculous or non-tuberculous, seldom show leucocytosis, unless there are complications. The blood examination does not distinguish between empyema and pneumonia.

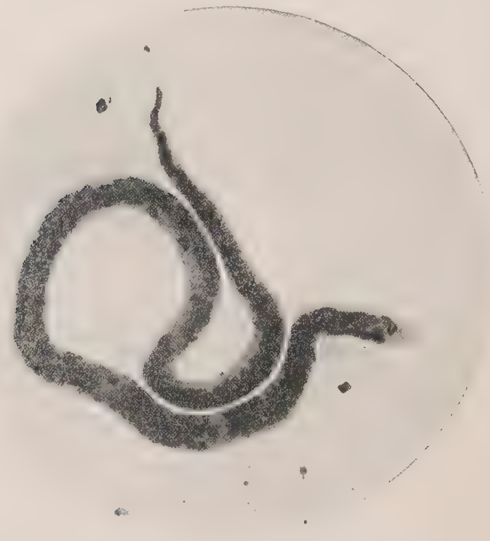
Endocarditis.—*Acute ulcerative endocarditis* shows a rapidly developing secondary anæmia with marked leucocytosis. The blood condition is that of acute septicæmia. The microörganism of the disease may often be grown in cultures from the blood, and if the first cultures prove negative repeated cultures usually prove positive. *Simple or benign acute endocarditis* differs pathologically from the malignant or ulcerative type only in degree and in the virulence of the infecting organisms, and, therefore, may or may not show leucocytosis, according to the severity of the infection, the variety and virulence of the microörganisms, and the associated affection to which the involvement of the heart valve is secondary, *e. g.*, rheumatism, tonsillitis, typhoid fever, etc. *Chronic valvular lesions* produce no changes in the blood, excepting as follows: When the blood pressure is lowered by acute failure of compensation the venous blood especially may show dilution, rarely the capillary blood. In partially compensated valvular lesion with chronic passive congestion (stasis) concentration of the blood may occur, especially in the capillary circulation. Such concentration seems to affect the leucocytes in some cases even more than the red corpuscles. Secondary anæmia is sometimes present, but may be masked by the concentration of the blood. Polycythæmia is less frequently seen with aortic than with mitral lesions. One must therefore be very guarded in drawing conclusions from the blood count in cases of valvular lesion not fully compensated. *Congenital valvular lesions*, with cyanosis, usually show concentration of the peripheral blood with increased red corpuscle and leucocyte counts.

Enteritis.—See Intestine.

Erysipelas.—A moderate leucocytosis with neutrophilia occurs in most cases, the count depending upon the severity of the infection. In mild cases leucocytosis is absent.

Exophthalmic Goitre.—The blood is usually normal. Some cases show leucopenia and relative lymphocytosis. The frequent association of *chlorosis* with Graves' disease may perhaps explain the latter condition. Eosinophilia has been occasionally observed.

FIG. 46.



Filaria sanguinis hominis, stained with methylene-blue.

Fecal Impaction.—See Intestines.

Filariasis.—The *Filaria sanguinis hominis* may be found in the peripheral blood, if the latter is examined at an appropriate hour. (See Fig. 46.)

Floating Kidney.—See Kidney.

Furunculosis.—The blood may show inflammatory leucocytosis, occasionally even from a single small boil.

Gall Bladder and Ducts, Diseases of.—*Cholangitis,* if simply *catarrhal,* is not accompanied by leucocytosis or increased fibrin, but when *suppurative* shows leucocytosis and increased fibrin.

Gall-stones in themselves cause no change in the blood. When *chronic jaundice* is induced by *obstruction of the ducts by stone* a secondary anæmia gradually develops, leucocytosis may occur, and retarded coagulation is very apt to be present.

Cancer of the bile ducts causes leucocytosis without increased fibrin in a majority of cases, and gradually developing anæmia.

Chronic obstructive jaundice, due to any kind of obstruction, leads to increased coagulation time, secondary anæmia, and (in some cases) leucocytosis. (See Liver.)

Glanders.—Leucocytosis with neutrophilia is said to occur.

Gonorrhœa.—Leucocytosis with neutrophilia and increased fibrin is the rule. Eosinophilia has been noted in some cases.

Gout.—Uric acid, as urates, is present in increased amount. Anæmia may develop in severe chronic cases. The leucocytes are said by some to be increased, by others to be normal. Perinuclear basophilic granules are seen most frequently in this disease, according to Neusser, and eosinophilia has been noted.

Grippe (Specific Influenza).—Leucocytosis is usually absent, but occurs in a small minority of cases. **Differential Diagnosis**: The absence of leucocytosis is valuable in excluding pneumonia.

Hæmophilia.—Secondary anæmia; no leucocytosis, except after severe hemorrhage, often leucopenia; coagulation time markedly increased; blood platelets much reduced in number.

Hemorrhage.—A single, small hemorrhage produces no change, while a single large hemorrhage causes secondary anæmia usually with leucocytosis. Repeated small hemorrhages produce the blood picture described under Hemophilia. The cause of the hemorrhage must always be considered. (See Plate XXXVI., Fig. 3.)

Hodgkin's Disease.—The blood picture of pseudoleukæmia lymphatica (Hodgkin's disease) is in general similar to that of pseudoleukæmia splenica (splenic anæmia), except that in the former the anæmia does not develop so early and seldom reaches so high a grade. Leucocytosis is rare and leucopenia is often seen. Nothing characteristic is seen in the differential leucocyte count. Myelocytes may occur occasionally, but only in small numbers. Lymphocytosis is sometimes seen. Occasionally cases are seen with some leucocytosis. Inflammatory leucocytosis may occur as the result of secondary infection and ulceration of the enlarged superficial glands.

Differential Diagnosis.—From *lymphatic leukæmia* it is easily distinguished by the high leucocytosis with high relative lymphocytosis, characteristic of lymphatic leukæmia. The blood examination is the only means of distinguishing Hodgkin's disease from leukæmia. The characteristic blood pictures of *spleno-myelogenous leukæmia* and *mixed lymphatic and spleno-myelogenous leukæmia* distinguish these diseases from the not uncommon cases of Hodgkin's disease with moderate splenic enlargement. A few cases of Hodgkin's disease have been seen to develop into true leukæmia, the blood picture changing characteristically. Such a case the writer has lately been enabled to follow in Buffalo, by the courtesy of Dr. Grover W. Wende. *Lymphosarcoma* can often be distinguished from Hodgkin's disease by the (usual) occurrence of a distinct leucocytosis in the former and its absence in the latter, but exceptions occur, causing difficulty in diagnosis. The blood examination is not decisive in distinguishing Hodgkin's disease from *glandular syphilis and tuberculosis.*

Hydatid Cyst.—See Echinococcus Cysts.

Hydronephrosis.—See Kidney.

Hypodermoclysis.—Subcutaneous (as well as intravenous) infusion of normal salt solution has been observed to rapidly increase the leucocyte count, and lower the red count.

Hysteria.—The blood is usually normal. Some cases show slight anæmia. The eosinophiles have been found increased moderately in some cases.

Icterus.—See Liver, also Gall Bladder and Ducts.

Infants.—See Anæmia, Secondary, Physiological Leucocytosis in Infancy and Children.

Inflammatory Processes.—If of long standing, a secondary anæmia develops. In general leucocytosis with relative neutrophilia and increased fibrin formation are present. (See Pathological Leucocytoses, Inflammatory, and Malignant Tumor; also Plate XXXVI., Fig. 4.)

Intestine, Diseases of.—Simple *diarrhœa* may show a transient polycythæmia from concentration. *Catarrhal enteritis* usually shows no change in the blood. *Acute ulcerative enteritis* shows concentration and leucocytosis. *Chronic ulcerative enteritis* shows secondary anæmia, sometimes leucocytosis. *Acute gastroenteritis in infants* usually shows concentration of blood and leucocytosis with relative lymphocytosis. *Intestinal obstruction* from any cause shows marked leucocytosis in the early stage, which disappears with the advent of *gangrene* (Bloodgood). *Fæcal impaction* shows no change. *Cancer* of the intestine is often without leucocytosis, perhaps due to its slow growth and small size. *Colic.* See Colic. *Intestinal parasites* (worms) sometimes cause marked eosinophilia. Müller and Rieder in two cases of *ankylostomiasis* found respectively 8.2 per cent. and 9.7 per cent. of eosinophiles. Zappert in two cases of the same disease found the eosinophiles increased up to 17 per cent., while in a third case there was no increase. Leichtenstern in another case found 72 per cent. of eosinophiles. Zappert and Leichtenstern both noted the presence of numerous *Charcot-Leyden crystals* in the feces of those cases in which they found eosinophilia, and the absence of such crystals in the feces of cases of the disease not showing eosinophilia. Bücklers found 16 per cent. of eosinophiles in a case infected with *Oxyuris vermicularis*, and 19 per cent. in a case infected with *Ascaris lumbricoides.* In a case of *Tænia mediocanellata* Leichtenstern found 34 per cent. of eosinophiles. (See also Trichinosis and Skin.)

Jaundice.—See Gall Bladder and Ducts, and Liver.

Kidney, Diseases of.—According to Cabot, "aside from uræmia and hæmorrhage, *nephritis* probably does not cause leucocytosis." Secondary anæmia usually develops. *Uræmia* is usually accompanied by leucocytosis. In the absence of uræmia, acute nephritis more frequently than chronic nephritis shows leucocytosis. *Pyelo-nephritis* usually shows anæmia and leucocytosis. *Hydronephrosis, floating kidney* and *simple cystic kidney* are all without blood changes. *Stone* in the kidney and *colic* from its passage show no change in the blood, unless considerable injury and ulceration have been caused, in which case leucocytosis develops. *Pyonephrosis* usually shows inflammatory leucocytosis with increased fibrin, as does *perinephritic abscess. Malignant tumor* of the kidney, whether carcinoma or sarcoma, is generally accompanied by distinct leucocytosis, without increased fibrin. **Differential Diagnosis**: Great difficulty is sometimes experienced in determining the organ involved in solid abdominal masses located in the left flank, *i. e., whether kidney or spleen.* Blood examination here comes to our assistance. The presence of marked and persistent leucocytosis without increased fibrin strongly favors malignant tumor, and, as malignant disease is many times more frequent in the kidney than in the spleen, an opinion of the existence of malignant tumor probably of the kidney may be advanced. On the other hand, the absence of leucocytosis or the presence of leucopenia favors non-malignant hypertrophied spleen. The application of this guide must be employed with great caution and only in conjunction with all the other evidence obtainable from a careful study of the case and a consideration of the history. Cases thus carefully studied occasionally occur in which the additional clew furnished by the blood is the deciding factor in diagnosis. The writer has applied this rule successfully for a correct diagnosis in six cases of malignant tumor of the kidney and several cases of hypertrophied spleen during the past two years, without failure in a single case. In this connection, it seems hardly necessary to say that the question of leukæmia would be removed at once by blood examination. *Syphilitic enlargement of the spleen* might cause embarrassment in diagnosis in some cases. *Hæmorrhagic infarction* of the spleen causes leucocytosis, but the history and symptoms make the diagnosis clear.

Lead Poisoning.—Chronic plumbism causes anæmia with degenerative changes in the red corpuscles, especially granular degeneration, which is a very valuable diagnostic sign in suspected cases. Leucocytosis is absent.

Leukæmia.—Both the lymphatic and myelogenous types of leukæmia show characteristic blood pictures. The leukæmias are among the few diseases that can be positively diagnosticated by blood examination alone.

Myelogenous or Spleno-myelogenous Leukæmia.—A gradually developing, moderate, secondary anæmia is present; degenerative changes in the red corpuscles correspond to the degree of anæmia. Nucleated red corpuscles, both normoblasts and megaloblasts, the former predominating, are very numerous, even in cases showing little anæmia. Fragmentation of the nuclei and even karyokinetic figures may be seen. The most massive leucocytosis known in any disease characterizes this affection, ranging from 100,000 to 1,000,000 or more, and averaging over 400,000 per cubic millimeter. But not so much the size of the leucocytosis as its peculiar differential quality characterizes the disease.

The essential point is the presence of a large proportion of myelocytes, constituting from 20 to 50 per cent. of all the leucocytes. Neutrophilic myelocytes constitute the great majority of the myelocytes, but occasionally cases are seen with frequent eosinophilic myelocytes. The myelocyte increase occurs at the relative expense of the polymorphonuclear neutrophiles and the lymphocytes, especially the latter. The neutrophiles average about 50 per cent., the lymphocytes about 10 per cent. The polymorphonuclear eosinophiles usually show a slight relative increase, but a wide variation sometimes occurs. Basophilic leucocytes are frequent. Mast-cells constitute a few per cent. of all the leucocytes in most cases of myelogenous leukæmia. Perinuclear basophilic granules are also very common. The leucocytes in general are very polymorphous, *i. e.*, the different kinds of leucocytes show a strong tendency to vary from the typical in size and form. Charcot-Leyden crystals may separate from the blood after standing. (See Plate XXXVI., Fig. 7.)

Lymphatic leukæmia.—Considerable anæmia with occasional nucleated red cells is the rule. In severe acute cases the nucleated reds may be numerous. The leucocytosis is high, but not usually so great as in myelogenous leukæmia. The leucocytes average from 100,000 to 150,000 per cubic millimeter. In exceptional cases the count may be much lower or remarkably high. *The differential leucocyte count shows a remarkable lymphocytosis, averaging over 90 per cent. of the leucocytes.* The other forms of leucocytes are reduced to very small percentages. Myelocytes are only rarely seen. Acute cases often show a predominance of the large form of lymphocyte over the small form. Wide variations in the size of the lymphocytes and their staining quality are seen, so that it is often impossible to draw a sharp distinction between the small and large lymphocytes, nor is it important to do so. The blood platelets are increased in both types of leukæmia. (See Plate XXXVI., Fig. 6.)

Mixed Leukæmia.—Cases are occasionally seen that show in the blood a mixture or fusion of the characteristics of the two pure types.

The effect of intercurrent infections on the blood of leukæmia is interesting. In Richter's case of acute articular rheumatism complicating leukæmia no change in the blood occurred. In Müller's case of septicæmia complicating lymphatic leukæmia the leucocytes were largely increased, with a great relative increase of the neutrophiles. These cases, however, may be regarded as exceptions. The rule is for intercurrent infections to greatly reduce the number of leucocytes, sometimes even to normal, with or without a relative increase of the neutrophiles. Preceding death from leukæmia the leucocytosis sometimes drops, and occasionally falls below normal.

Differential Diagnosis.—*Inflammatory leucocytosis* is occasionally so great that a diagnosis of leukæmia might be made if only the number of the leucocytes were considered, but the differential quality of the leucocytosis makes the distinction easy. All other affections are readily distinguished from leukæmia by the absence of the characteristic blood changes of the latter.

Löwit has recently claimed to have discovered *protozoa* in the leucocytes in leukæmic blood, and believes them to be the infective agent of the disease. This claim has been sharply combated by Türk and Kraus, who consider the bodies described by Löwit to be merely basophilic granules in the mast-cells, so frequent in leukæmic blood.

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Türk and *Kraus.* See discussion before the Eighteenth German Congress for Internal Medicine, held at Wiesbaden, April 18–21, 1900.

Liver, Diseases of.—*Abscess* shows the blood picture of abscess of any part of the body.

Jaundice of any variety shows bile pigments in the serum, which may interfere with the estimation of hæmoglobin. The serum is colored yellowish (bilirubin), which on heating turns to green (biliverdin). *Catarrhal jaundice* is usually without change in the formed elements of the blood. The absence of persistent leucocytosis is important. Increased size of the red corpuscles has been observed by von Limbeck.

Chronic obstructive jaundice.—See Gall Bladder and Ducts.

Cholangitis.—See Gall Bladder and Ducts.

Cirrhosis (*atrophic*) as a rule shows anæmia without leucocytosis. After hemorrhages leucocytosis may occur.

Cirrhosis (*hypertrophic*) may show secondary anæmia. Hayem records a plus color-index in one case. Leucocytosis often occurs.

Icterus neonatorum.—There is nothing characteristic in the fresh blood. [Post-mortem blood clots show hæmatoidin crystals in abundance.]

Malignant tumors.—*Carcinoma* shows anæmia and in a majority of cases leucocytosis without increased fibrin. The leucocytosis, however, in many cases is absent. *Sarcoma,* especially melanosarcoma, more constantly than carcinoma causes leucocytosis.

Syphilis of the liver follows the rule of syphilis in general.

Malaria.—The red corpuscles and hæmoglobin are reduced with each paroxysm, generally about equally. Between attacks the red cells gain more rapidly than the hæmoglobin. In pernicious malaria the anæmia is extreme, with proportionate degenerative changes. The blood platelets are reduced in number. The leucocytes are normal in number or subnormal (usually). When leucopenia exists, lymphocytosis is usually found, with the large lymphocytes increased relatively to the small lymphocytes. In a few cases of grave postmalarial anæmia (Billings) and comatose pernicious malaria (Kelch) a temporary leucocytosis has been observed. Leucocytosis, however, is to be considered a rare exception, and leucopenia is the rule in all ordinary malarial infections. Leucocytes containing granules of malarial pigment (melaniferous leucocytes) and even entire malarial parasites are frequent in malarial blood.

Malarial parasites are best studied in the fresh-blood preparation. Three varieties of organisms are found, the *tertian, quartan,* and *æstivo-autumnal,* producing respectively the three different types of malarial fever bearing the same names. (See Plate XXXVII.) The febrile paroxysm is caused by the maturing and segmentation of a group of organisms, and a few hours before or after the paroxysm is the best time for finding the parasites in the blood. A few

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DESCRIPTION OF PLATE XXXVII.

Malaria. Figs. 1–6 from a case of double tertian infection; Figs. 7–12 from cases of æstivo-autumnal infection. Stained with methylene-blue and eosin.

FIG. 1. On the left two corpuscles, containing each a single, young, annular, hyaline parasite; on the right an expanded corpuscle, containing a large, pigmented, hyaline parasite, twenty-four hours older than the two small parasites.

FIG. 2. Parasite about one-third grown.

FIG. 3. Pigmented parasite about two-thirds grown.

FIGS. 4–6. Segmenting parasites. The shadow of the corpuscle is still seen about the young spores in Fig. 4.

FIG. 7. Young hyaline body, showing a ring shape.

FIGS. 8–12. Crescents and ovoid bodies, with the pigment granules clustered at the center. Figs. 8–10 show the "bib" or remnant of the corpuscle attached to the parasite on one side.

PLATE XXXVII.

Fig. 1.

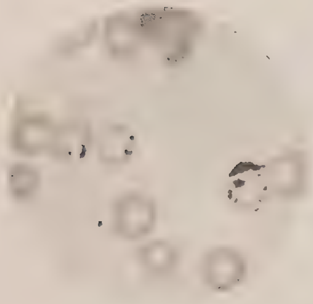


Fig. 2.

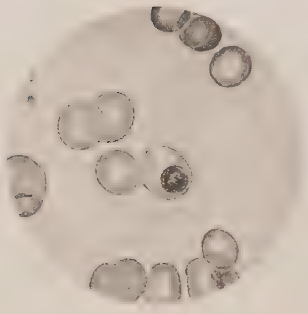


Fig. 3.

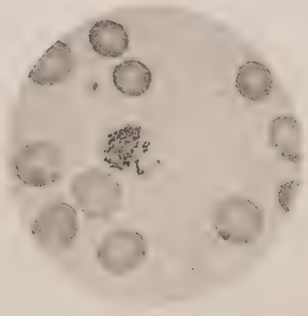


Fig. 4.

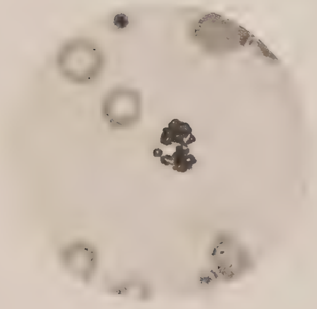


Fig. 5.

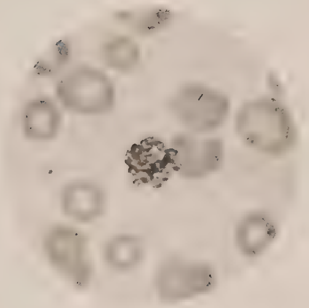


Fig. 6.

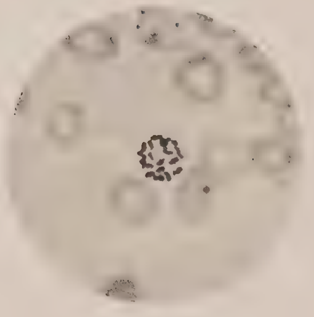


Fig. 7.

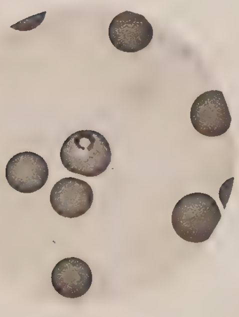


Fig. 8.

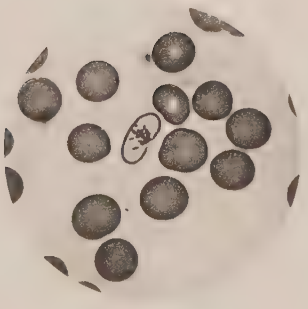


Fig. 9.

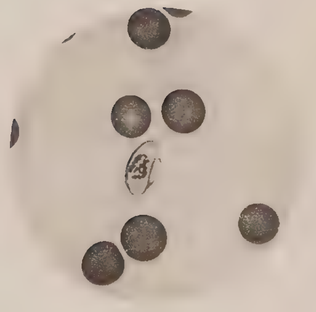


Fig. 10.

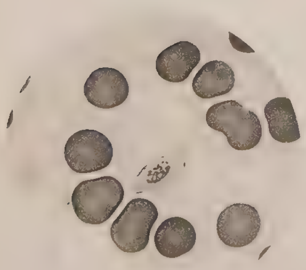


Fig. 11.

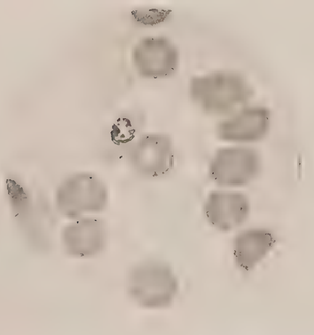
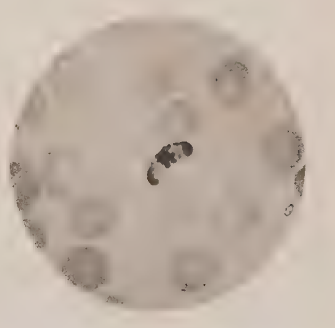


Fig. 12.



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minutes' search is usually sufficient for discovering the parasites in the blood in tertian and quartan infections, but repeated examinations may be necessary in æstivo-autumnal fever, especially near the time of the paroxysm, as full-grown and segmenting æstivo-autumnal parasites are rarely found in the peripheral circulation. It may be mentioned that Marchiafava and Bignami and others have claimed to distinguish two varieties of the æstivo-autumnal organism, maturing respectively in about twenty-four and forty-eight hours, and characterized by structural differences, as well as by the types of the temperature curves produced. Most observers have failed to recognize two varieties of this organism. A description of the different phases of the growth of the three malarial organisms is beyond the scope of this chapter and the student is referred to the following special treatises:

Differential Diagnosis.—Finding the malarial parasites in the blood is positive in the diagnosis of malaria, but this does not exclude the possibility of *coincident infections,* such as *typhoid fever* and *pneumonia.* Typhoid fever shows about the same general blood picture, excepting the parasites, as does malaria. The Widal test would be decisive. The presence of inflammatory leucocytosis and increased fibrin with physical signs suggestive of pneumonia would render the diagnosis of pneumonia probable. The leucopenia of ordinary malarial infections is one of the most important differential points in excluding suppurative processes. The types of the organism found differentiate the types of malarial infection. Combined malarial infections are common, such as double tertian, double or triple quartan and tertian and æstivo-autumnal. *The diagnosis of malaria should never be made upon clinical or therapeutic data,* and the organisms should always be found in support of the diagnosis. Tuberculosis with hectic fever, typhoid fever and various inflammatory processes, such as puerperal sepsis, are commonly diagnosticated as malaria in the absence of blood examination. The blood examination suffices to avoid such errors. It must be remembered that vacuoles and deformities in the red corpuscles are frequently mistaken for malarial organisms by those who have not had special experience with malarial blood, and caution in this respect is therefore enjoined.

Malignant Tumor (Carcinoma and Sarcoma).—Both carcinoma and sarcoma show a gradually developing anæmia, which may become profound and simulate pernicious anæmia. The fibrin formation is normal or subnormal unless ulceration or inflammation occurs. The observation of this point is of some practical value. Leucocytosis is the rule, generally more marked in sarcoma than in carcinoma; but many exceptions occur. The presence of leucocytosis is of more significance than its absence in a case of suspected malignancy. (See Leucocytosis of Malignant Tumor, under Pathological Leucocytoses; also, **Differential Diagnosis**, under the different organs.) *Malignant leucocytosis is differentiated from inflammatory leucocytosis, in general, by the absence of increased fibrin in the former and its presence in the latter.*

Malta Fever.—A gradually developing anæmia, absence of leucocytosis and the occurrence of a clump reaction characterize this disease.

Measles.—The blood is usually normal. A progressive leucopenia develops in some cases. **Differential Diagnosis**: The absence of leucocytosis excludes scarlet fever, as well as diphtheria. German measles (rötheln) is also without leucocytosis.

Meningitis.—See Brain, and Cerebro-Spinal Meningitis (Epidemic).

Mumps.—Cabot's five cases showed absence of leucocytosis. (See Parotitis.)

REFERENCE:

Thayer. Lectures on the Malarial Fevers. New York, 1897.

Marchiafava and Bignami. Malaria. Twentieth Century Practice of Medicine, Vol. XIX., pp. 3–522. New York, 1900.

Nephritis.—See Kidney.

Neuralgia.—Normal blood.

Neurasthenia.—Normal blood.

Neuritis.—Febrile multiple neuritis (one case) showed high leucocytosis. Afebrile alcoholic neuritis in a majority of cases showed moderate leucocytosis. Post-diphtheritic neuritis (one case) was without leucocytosis. Neuritis from chronic plumbism was without leucocytosis in twenty-five cases. (Cabot's statistics.)

Œsophagus, Diseases of.—Simple *non-malignant stricture,* if severe enough to cause starvation of liquids, shows leucopenia and concentration of the blood. *Malignant (cancerous) stricture* usually shows the same blood changes, but leucocytosis occurs in some cases. The difference in the leucocyte counts probably depends upon the size and rate of growth of the cancer, as well as upon the presence or absence of metastases.

Otitis Media.—If *purulent,* leucocytosis is the rule. If *catarrhal or serous,* leucocytosis is rarely found. *Mastoiditis or sinus infection,* complicating, raises the leucocyte count. Chronic cases may show moderate anæmia.

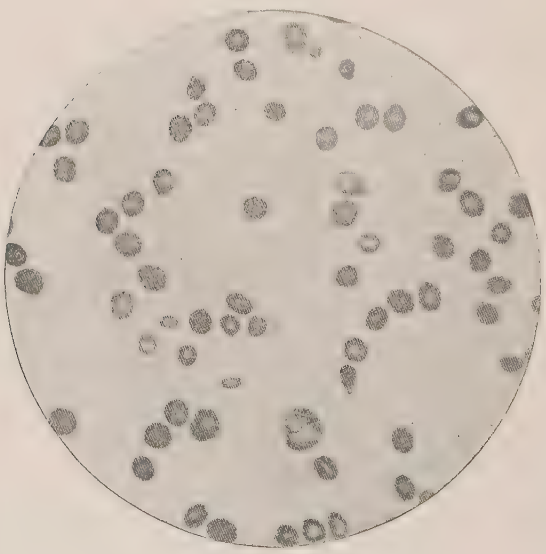
Osteomyelitis.—If purulent and without discharging sinus, leucocytosis with increased fibrin is present.

Parasites.—See Echinococcus Cysts, Filariasis, Intestine, Leukæmia, Malaria, Relapsing Fever, etc.

Parotitis.—*Acute epidemic parotitis.* (See Mumps.)

Symptomatic parotitis, occurring as a complication of the infectious fevers, facial paralysis, etc., and following injuries, diseases, and operations of the abdominal and pelvic organs, tends to become suppurative and is accompanied by leucocytosis and increased fibrin.

FIG. 47.



Pernicious anæmia.

Pericarditis. — *Rheumatic and septic pericarditis* are almost invariably accompanied by marked leucocytosis. *Tuberculous pericarditis* is without leucocytosis. **Differential Diagnosis**: Given a pericarditis, the absence of leucocytosis argues for its tuberculous nature.

Peritonitis. — *Tuberculous peritonitis* rarely shows leucocytosis. Young children may show exception to this rule. Other forms of peritonitis, purulent and serous, show marked leucocytosis with increased fibrin. Cases of overwhelming septic peritonitis may be without leucocytosis, following the general rule in such cases. (See Inflammatory Leucocytosis under Pathological Leucocytoses.) *Localized abscess,* as in appendicitis, pelvic peritonitis, etc., follow the general rule and show leucocytosis. **Differential Diagnosis**: Except in acute septic peritonitis, the absence of leucocytosis usually indicates the tuberculous nature of the process.

Pernicious Anæmia, Progressive (Primary).—The anæmia is profound. The red corpuscles are reduced to very low figures, often below one million, and average about a million and a quarter per cubic millimeter. Marked degenerative changes are constant, poikilocytosis, granular degeneration, polychromatophilia, etc., and nucleated red corpuscles are very frequent, *the megaloblasts predominating over the normoblasts.* In no other condition are nucleated red corpuscles so numerous. The size of the red cells varies markedly. *The average size is increased above the normal.* The hæmoglobin is greatly reduced, but in a majority of cases not in proportion to the reduction in the number of corpuscles, so that *a relatively high color-index, usually plus, is the rule.* The average amount of hæmoglobin is from 30 to 40 per cent. *Leucopenia* characterizes the disease, the reduction in the number of the leucocytes progressing with the disease. Very low leucocyte counts are common. Leucocytosis is never seen excepting with complications. Lymphocytosis characterizes the differential leucocyte count. A small percentage of myelocytes is usually found. Fibrin and platelets are reduced. (See Fig. 47.)

Differential Diagnosis.—The high color-index, the increased average size of the red cells, the frequence of nucleated cells with megaloblasts predominating, and the leucopenia differentiate the disease from extreme *secondary anæmia. Malignant tumor,* causing such marked anæmia as to simulate pernicious anæmia, is usually accompanied by marked leucocytosis with increased neutrophilia. Megaloblasts and megalocytes are infrequent in malignant cachexia, as opposed to pernicious anæmia. Between *chlorosis* and pernicious anæmia, no doubt could arise, as the blood pictures are widely different.

Phlebitis.—Septic phlebitis is accompanied by leucocytosis and increased fibrin. Phlebitis and venous thrombosis, complicating typhoid fever, also show leucocytosis. Whether the so-called marantic thrombosis of debilitating diseases and states of blood impoverishment is accompanied by leucocytosis or not remains to be worked out. There is, however, some reason for thinking that leucocytosis is the rule in such processes.

Phosphorus Poisoning.—Concentration of the blood may be marked. The leucocytes are markedly increased in some cases, in others normal or subnormal.

Phthisis.—See Tuberculosis.

Pleurisy.—Morse concludes from 224 counts made on 20 cases of *serous pleurisy,* including tuberculous and non-tuberculous cases, that primary serous pleurisy without complications is without leucocytosis. This result agrees closely with Cabot's findings in 99 cases. For *purulent pleurisy* see Empyema. **Differential Diagnosis**: From empyema, pneumonia, and malignant growths in the pleura, by the leucocytosis of the latter.

Pneumonia.—Marked leucocytosis with increase of fibrin is the rule. Exceptions occur in the mildest and most virulent and fatal cases. (See Inflammatory Leucocytosis under Pathological Leucocytoses.) The leucocytosis appears at the onset of the disease and persists throughout. It falls rather abruptly at the time of the crisis and disappears within a day or two after. Pseudo-crisis does not influence the leucocyte count. The leucocytosis fades gradually when the termination is by lysis instead of crisis. In delayed resolution the leucocytosis persists. A very high leucocytosis is a bad sign. Leucocytosis below 20,000 or 25,000 may be regarded complacently, as the normal expression of the disease. *In cases of pneumonia showing signs of unusual severity the absence of leucocytosis indicates usually a fatal termination.* The leucocytosis shows a high percentage of neutrophiles. Marked increase in the fibrin network is seen in nearly all cases showing leucocytosis. A moderate secondary anæmia may develop late in the disease. In a considerable proportion of cases cultures from the blood are positive, showing a growth of the pneumococcus. **Differential Diagnosis**: The presence of marked leucocytosis and fibrin increase argues strongly for pneumonia as opposed to grippe, ordinary bronchitis (see Bronchitis), and typhoid fever and malaria with pulmonary symptoms at onset. Masked and central pneumonias in alcoholics and the aged are indicated by the same means.

Pseudoleukæmia.—See Anæmia Splenica and Hodgkin's Disease.

Puerperal Septicæmia.—Marked leucocytosis with increased fibrin occurs in all cases, excepting those of overwhelming virulence, thus following the general rule of inflammatory leucocytosis.

Purpura.—The blood changes depend upon the nature and cause of the purpura, which is only a symptom. If severe hemorrhages occur, a marked anæmia and leucocytosis usually develop. The blood plates may be reduced and the coagulation time may be greatly increased. In *idiopathic purpura hemorrhagica* the leucocytes are normal or reduced in number, except after large hemorrhages, and relative lymphocytosis is usually found.

Purulent Processes.—Inflammatory leucocytosis with increased fibrin is the rule. (See Inflammatory Leucocytosis under Pathological Leucocytoses, Abscess, etc.)

Pyæmia.—See Purulent Processes, etc.

Pyelonephritis, Pyonephrosis, etc.—See Kidney.

Relapsing Fever.—The spirillum of Obermeyer is found in the blood following a paroxysm for about two days and is pathognomonic of the disease. In suspected cases occurring during an epidemic the blood may be tested for the reaction of Löwenthal. Anæmia may develop and leucocytosis is said to occur during and following the paroxysm.

Rheumatism.—*Acute Articular Rheumatism.*—Secondary anæmia, sometimes marked, usually develops. The hæmoglobin averages about 65 per cent. Leucocytosis is well marked and seen in nearly all acute cases. The neutrophiles are relatively increased. Fibrin is greatly increased.

Subacute Articular Rheumatism.—Anæmia; the leucocyte count varies according to the manifestations of the disease, most cases showing little or no increase.

Chronic articular rheumatism shows usually normal blood, except for a slight leucocytosis during exacerbations.

Muscular rheumatism, so called, shows normal blood.

Complications may, of course, greatly modify the blood picture.

Rickets.—Secondary anæmia is usual, sometimes of extreme grade. The leucocytes may not be increased in mild cases, but in a majority of cases leucocytosis with relative lymphocytosis is seen. Eosinophilia occurs in some cases. The blood changes are perhaps not due to any specific reaction in the disease, but rather to the young age of most cases and the tendency in childhood in conditions of debility to revert to the blood type of infancy. (See Leucocytosis of Infancy under Physiological Leucocytoses.)

Rötheln.—Normal blood. **Differential Diagnosis**: The absence of leucocytosis in German measles distinguishes the disease from scarlet fever. The blood examination does not assist in distinguishing German measles from measles.

Sarcoma.—See Malignant Tumor.

Scarlet Fever.—Slight secondary anæmia. Leucocytosis, usually well marked, is almost constant, appearing before the eruption and lasting until toward the end of the febrile period in most cases. The neutrophiles show an increase at the expense of the lymphocytes. A moderate increase of the eosinophiles is also seen in all except the severest cases, persisting well into convalescence. **Differential Diagnosis**: The leucocytosis differentiates scarlet fever from measles and German measles.

Scurvy.—Secondary anæmia. In uncomplicated cases, leucocytosis is not found. With marked hemorrhages or inflammatory complications leucocytosis may be marked.

Skin, Diseases of.—Eosinophilia has been observed in certain isolated cases of the following affections: *dermatitis herpetiformis, eczema, lupus, pellagra, pemphigus, psoriasis, scleroderma, urticaria*, etc.

Smallpox.—Marked secondary anæmia in severe cases. Leucocytosis occurs during the pustular eruption. Very mild cases may show no leucocytosis.

Spinal Cord, Diseases of.—Most of the chronic processes involving the spinal cord show nothing characteristic in the blood.

Spleen, Diseases of.—See Anæmia Infantum Pseudoleukæmica (von Jaksch), Anæmia Splenica, Hodgkin's Disease, Kidney (differential diagnosis), Leukæmia, etc. It must be remembered that splenic enlargement is secondary to many acute and chronic affections and especially to the anæmias of infancy. Syphilis and rickets in infants may produce great splenic hypertrophy. The blood condition depends upon the nature of the primary affection and the degree of the secondary anæmia.

Stomach, Diseases of.—*Acute gastritis* often shows moderate leucocytosis. The blood is otherwise normal.

Chronic gastritis may lead to secondary anæmia, sometimes very profound, but leucocytosis is absent. Digestion leucocytosis occurs in most cases, but not in all.

Carcinoma of the stomach in the early stages rarely shows leucocytosis, but when the disease is more advanced or when metastases exist leucocytosis is the rule. *Digestion leucocytosis is absent in most cases.* When leucocytosis occurs with gastric carcinoma, the neutrophiles are increased and the fibrin is normal or subnormal. Secondary anæmia is usually seen.

Ulcer of the stomach, with hemorrhage, leads to anæmia, sometimes grave. Leucocytosis is seen only after severe hemorrhages and during digestion.

Differential Diagnosis: In chronic stomach trouble the presence of leucocytosis, except after hemorrhage and digestion, suggests cancer. The absence of leucocytosis does not exclude carcinoma. The absence of digestion leucocytosis points to cancer, but does not exclude chronic gastritis.

Syphilis.—A secondary anæmia develops early in the disease and progresses in neglected cases to a severe grade. According to Justus' observations on three hundred cases, the administration of mercury by inunction or subcutaneous injection *during the primary stage* causes a sudden fall (within twenty-four hours) of hæmoglobin, ranging from ten to twenty per cent., which loss is gradually regained in a few days under treatment. Justus claims that this reaction to mercury is not observed in other diseases or after the occurrence of secondaries. Other observers (Brown and Dale, Christian and Foerster) have discredited this test as unreliable and occurring in other diseases as well as in the later stages of syphilis. The leucocytes are usually normal in number in the primary stage but are increased above normal during the eruption. In the later stages a slight leucocytosis is often seen. In all stages the differential count shows a lymphocytosis, which tends to be reduced by treatment with mercury and iodides. A moderate increase of eosinophiles is often observed in the secondary and ter-

REFERENCES:

Justus. Verhandl. d. 5 Congress d. Deutsch. Dermatolog. Gesellsch., September, 1895.
Brown and Dale. Cincinnati Lancet-Clinic, March 24, 1900.
Christian and Foerster. University Med. Magazine, Phil., November, 1900.

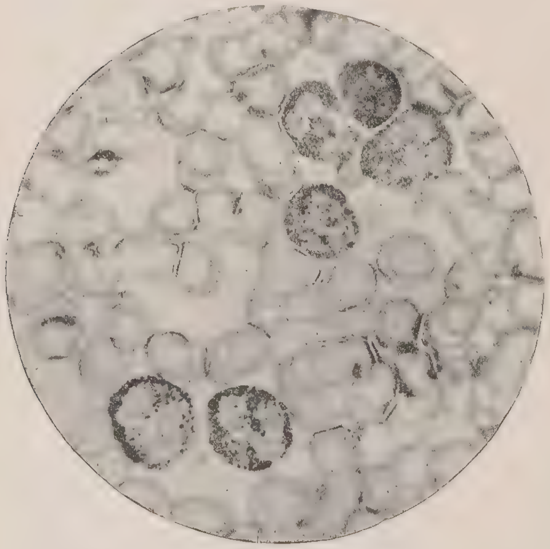
tiary stages. Myelocytes, in small percentage, may also be found in the late stages. Congenital and chronic late syphilis usually show anæmia, sometimes leucocytosis, with relative lymphocytosis and small percentages of myelocytes and a slight increase of eosinophiles.

Differential Diagnosis.—The occurrence of anæmia and moderate leucocytosis, with lymphocytosis, slight eosinophilia, and the presence of myelocytes, and without fibrin increase is suggestive of syphilis in obscure cases in adults. In children the same blood condition may arise from a variety of causes.

Tonsillitis.—*Follicular tonsillitis* is usually accompanied by a distinct leucocytosis. **Differential Diagnosis**: The blood examination is of no value in deciding between ordinary follicular tonsillitis and diphtheria. It is important to exclude the tonsils as a source of infection in cases showing unaccountable leucocytosis.

Trichinosis.—As first observed by Brown, from Osler's clinic, and later confirmed by many observers (Gwyn, Osler, Cabot, Atkinson, Stump, Blumer and Neuman, etc.), trichinosis is characterized by a *leucocytosis with high eosinophilia.* (See Fig. 48, and Plate XXXVI., Fig. 5.) In hardly any other affection are the eosinophiles so numerous, constituting from 20 to 70 per cent. of all the leucocytes. Eosinophilia appears early in the affection and persists for several months. **Differential Diagnosis**: The occurrence of high eosinophilia in obscure cases with chronic muscular stiffness and pains makes the diagnosis of trichinosis very probable and excludes rheumatism. *Intestinal worms* and certain *skin affections* occasionally cause very high eosinophilia and must be excluded before the diagnosis of trichinosis is affirmed. (See Intestine; and Skin.)

Fig. 48.



Trichinosis, showing the characteristic eosinophilia. (Specimen kindly furnished by DR. THOMAS R. BROWN.)

Tuberculosis. — Cabot's statistics show the following facts: In pure tuberculosis, involving any of the organs of the body except the meninges, no leucocytosis is found. Tuberculous meningitis usually, but not invariably shows a leucocytosis. Relative lymphocytosis is the rule in pure tuberculosis. There is no increase of fibrin. A mild secondary anæmia may occur, but as a rule the red cells are little affected and the hæmoglobin is only moderately reduced. Tuberculous abscess ("cold abscess"), unless secondarily infected with pyogenic organisms, also fails to show leucocytosis, thus differing from other abscesses. Secondary septic infection of tuberculous lesions leads to inflammatory leucocytosis with increased fibrin.

Tumors.—Benign growths of all kinds show normal blood. (See Malignant Tumor.)

REFERENCE:

Brown. Johns Hopkins Hosp. Bull., 1897, Vol. VIII., p. 79; also, Journal of Experimental Medicine, 1898, Vol. III., No. 3.

Typhoid Fever.—Secondary anæmia develops in the late stages of the disease. The leucocytes at first are normal and later gradually diminish in number, with a relative increase in the lymphocytes at the expense of the neutrophiles. The lymphocytes may outnumber the neutrophiles late in the disease. The large lymphocytes are increased relatively to the small lymphocytes. Fibrin is normal in amount. With septic complications, such as peritonitis from perforation, parotitis, phlebitis, and otitis media purulenta, inflammatory leucocytosis with increased fibrin appears, unless the vital reaction of the patient is low and the septic process virulent, as in some cases of general peritonitis following perforation. The writer has observed two such cases without leucocytosis. But the absence of leucocytosis in such cases must be regarded as exceptional. Cases of apparently uncomplicated typhoid rarely occur showing leucocytosis. Cabot mentions such a case. The Widal clump reaction is present in over 95 per cent. of cases of typhoid at some time during its course and should be repeatedly looked for until found in suspected cases. Bacteriological cultures made from the blood for diagnosis prove positive in a majority of cases of typhoid fever, when the newer technique above described under Bacteriological Cultures is followed out. This method of diagnosis is especially valuable in the first week or ten days of the disease and is often positive in advance of the development of the Widal reaction. **Differential Diagnosis**: The presence of the Widal reaction is decisive. The absence of leucocytosis and the presence of relative lymphocytosis suggest typhoid fever, but a similar blood picture occurs in malaria, miliary tuberculosis, and other diseases. Septic processes are excluded by the low leucocyte count. The advent of marked leucocytosis in the course of typhoid fever indicates a septic complication. The application of this rule is of great surgical value in suspected perforation.

Typhus Fever.—Progressive secondary anæmia, without leucocytosis, has been described in a few cases.

Uræmia.—See Kidney.

Vaccinia.—Sobotka in forty-three cases found a well-marked leucocytosis occurring between the third or fourth day and the seventh or eighth day, succeeded by a normal or subnormal count, with a secondary leucocytosis developing on the tenth or twelfth day and lasting only a few days.

Whooping Cough.—Marked or very high leucocytosis, appearing early before the cough and persisting throughout the disease; relative lymphocytosis.

Yellow Fever.—Leucocytosis is marked in some cases, in others absent.

PART III.

THE PRINCIPLES OF OPTICS AND PHOTOMICROGRAPHY.

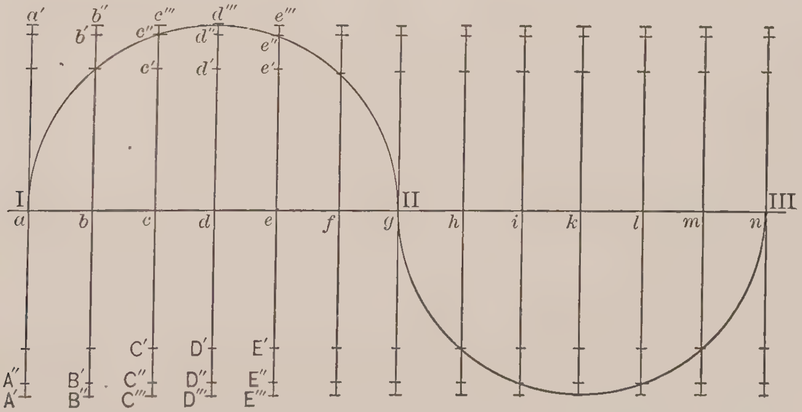
THE majority of text-books which deal with the optical problems relating to the microscope, are usually so technical in character as to be of little service except to those who possess an extensive knowledge of mathematics. For this reason it has been deemed advisable to preface this portion of our subject with a short résumé of the optical principles with which the student must be acquainted in order that he may understand the more complicated problems of photomicrography.

The foundation of all of our knowledge of refraction, reflection and transmission of light is based upon a theory which conceives of the propagation of light in straight lines by means of the vertical movements of atoms of æther.

The term *æther* is a name arbitrarily applied to a substance which is supposed to permeate all other substances. It has certain definite characteristics expressed by phenomena, one of which is the transmission of light. Within recent times it has been shown that other phenomena, such as the transmission of the Roentgen ray, that of the Marconi telegraph, and possibly also the transmission of electricity, presumably occur through the medium of this all-pervading substance.[1]

In order to understand the propagation of light, it is necessary to refer to the diagram (Fig. 49). The line I, II, III represents the direction in which the

FIG. 49.



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[1] Sound is transmitted in direct lines by the vibrations of molecules of air set in motion by the direct contact or impulse of one molecule against the other starting from the center where the sound occurs.

The diagrams used in this chapter are largely drawn from a small book by Prof. Miethe, entitled, Photographische Optik ohne Mathematische Entwickelungen, Berlin, 1893. To those who desire further elucidation of the points touched upon, this work is heartily recommended. Kaiserling's Compendium der Wissenschaftliche Photographie is also warmly recommended.

light is propagated. An atom of æther set in motion by stimulation from a source of light, being a vertical vibration which carries it upward to the point a'; in so doing it has attracted the molecule b, which is carried upward to the point b' and which in its turn has attracted the molecule c, which is attracted upward to the point c'; c attracts d, d attracts e, etc. In the meantime, the atom a has left a' and has begun to descend toward the point a. When it has reached a point indicated by the first cross line in the diagram, the atom b at b' has reached the point b''; the atom c at c' has reached the point c''. When the atom b has reached b'' it begins to descend; a has by this time reached the second cross line in descent; c'' ascends to c''' and begins its descent; d does the same; e, f and g likewise, and so on indefinitely. When a has reached the point a from which it started, it does not stop, but passes across the median line to a point A′, equally distant from a, and at this point the various phenomena relating to b, c, d, etc., are repeated. It will be seen from the diagram that if all the atoms could suddenly be fixed in their movements a line from a passing through each atom would describe an ascending curve from a to d'''. Here it would begin to descend, passing to the point II, where it would pass below the median line, describing a similar curve, and would return to the median at III. By such means the light is transmitted in the direction of I, II, III.

The rate of transmission of light varies with the density of the medium through which it passes. Through a rare medium it is transmitted at a quicker rate than through one more dense. It is also transmitted in all directions; that is, from a given source rays of light pass out in all directions.

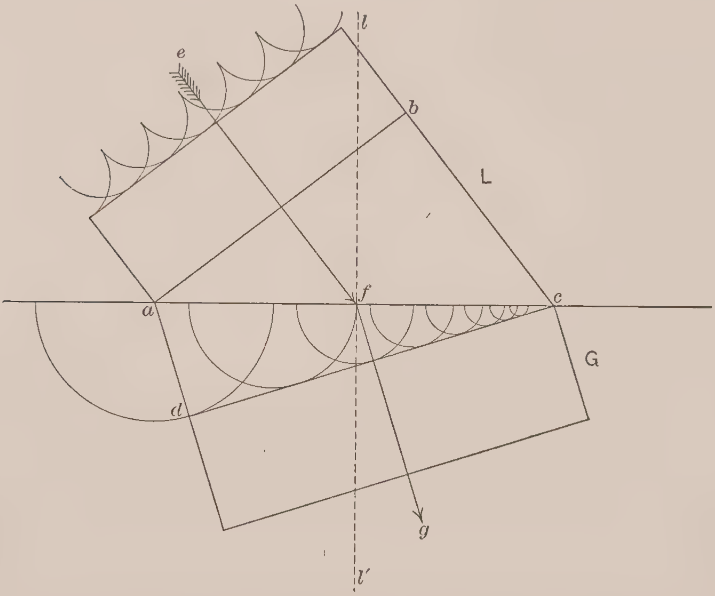
This is the so-called wave motion, which is identical in all respects to that occurring in water or other fluids where waves are propagated along the surface. It will be seen by the diagram that the length of the wave is the distance I–II. The height or amplitude of the wave is shown by the line d–d'''. This form of transmission is known as transverse wave motion, in contradistinction to longitudinal wave motion, by which sound is propagated.

We see, therefore, that a ray of light is transmitted by transverse wave motion, and as the wave has a distinct amplitude, we may conceive of the ray of light having definite width. It is necessary to conceive of this phenomenon of width in order to understand the next quality of light which concerns us—that of refraction. If a ray of light (Fig. 50) transmitted in space along the line ef in the direction indicated by the arrow, meets a plane transparent surface at c, such as a surface of glass, it changes its direction, or is refracted, toward the perpendicular ll'. We may conceive of the ray of light having the breadth ab. As the light is propagated along the line ef, one edge of the ray of light strikes the glass at a, while the opposite edge at b has still to travel the distance bc before coming in contact with the glass surface at c. The portion of the ray which has entered the substance of the glass at a is delayed in its transmission, because, as we have learned, light is not transmitted with the same rapidity through a dense medium as through a rare; it has, therefore, traveled only the distance ad during the time that the opposite edge b has occupied in reaching the point c.

The ray of light when it strikes the surface of the glass, as indicated in the diagram, transmits vibrations from the point of impact in all directions in the glass. When the ray has reached the point c at the surface, each atom in succession, beginning with a, has passed into the substance of the glass and within it has transmitted vibrations from the point of impact in all directions, with a result that may be described by a series of circles, as indicated in the diagram. If, then, we draw a line touching the surface of all the circles which represent the distances to which the atoms traveling in the same direction have transmitted vibration, we have the line dc, and if we erect a perpendicular to this line touching the glass surface at f where the line ef, which represents the direction of transmission of the ray of light, ends, it gives us the new axis of the ray fg, which we see by the diagram has changed its direction, falling nearer to the perpendicular ll'.

This phenomenon of the change in direction of a ray of light passing from one medium into another, is the fundamental basis of all the optical problems involved in the construction of the microscope. The angle of refraction is the angle $l'fg$; the angle of impact is the angle efl. The relation between the sine of the angle of impact and the sine of the angle of refraction of two substances is always constant. The refractive exponent of any substance is the expression in figures of the number of times which the angle of impact is greater than the angle of refraction. When the ray of light passes from air into some other substance indicated, the refractive exponent or index of air is taken at 1.00. From this we may figure the refractive index of various other substances, of which a short list is hereby appended:—

Fig. 50.



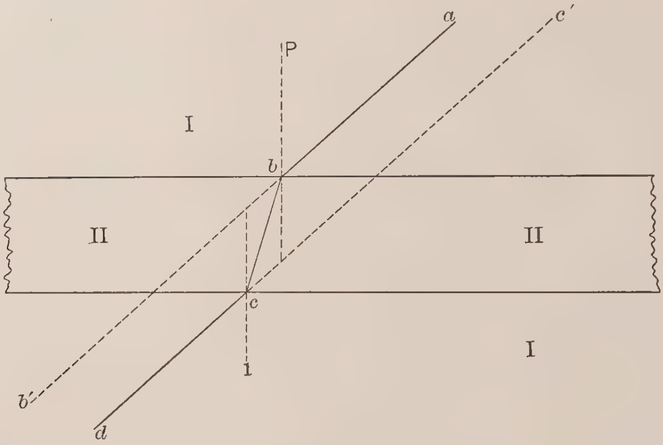
Air,	1.00	
Water,	1.33	
Alcohol,	1.37	
Crown Glass,	1.50–1.57	relatively
Flint Glass,	1.56–1.80	
Diamond,	2.47	

In the diamond it will be seen that the sine of the angle of impact would be nearly $2\frac{1}{2}$ times greater than the sine of the angle of refraction.

It will be seen that by this means, in combination with mathematical calculations, it is possible for the optician to determine accurately the direction which a given ray of light will take in passing from a given substance into any other given substance.

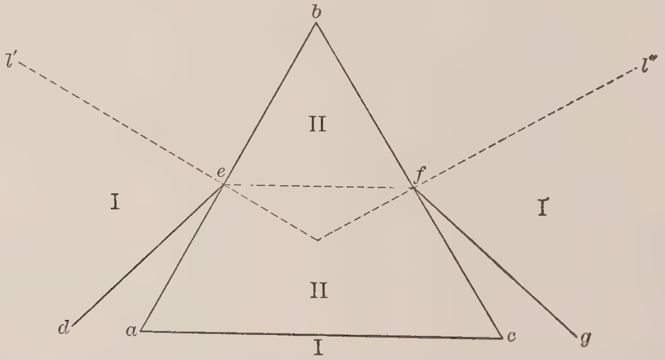
Fig. 51 illustrates the course of a ray of light ab, which strikes the surface of a plate of glass having parallel sides. The ray of light impinges at *b*, is refracted, as we have seen, toward the perpendicular which may be erected at *b*, passes downward and leaves the opposite surface of the glass at *c*, where by reason of its passing from a denser into a rarer medium, the phenomenon is reversed and it is refracted away from the axis exactly to the same extent to which it was refracted toward the axis in passing from the air into the glass. This we know must be the case, as the relation of the sines of the angle of impact

FIG. 51.



and the angle of refraction is constant. Therefore the angle of exit, *dcl''*, is equal to the angle of impact *pba*. But we shall see by the diagram that the ray of light transmitted in the direction *abb′* has not passed through the glass in a straight line, otherwise it would have followed the direction *bb′*, but by being refracted toward the axis it has left the glass at the point *c*, and being refracted away from the axis on passing from the denser to the rarer medium, it has taken the direction *cd*, which, we observe, is parallel to *ab*. In other words,

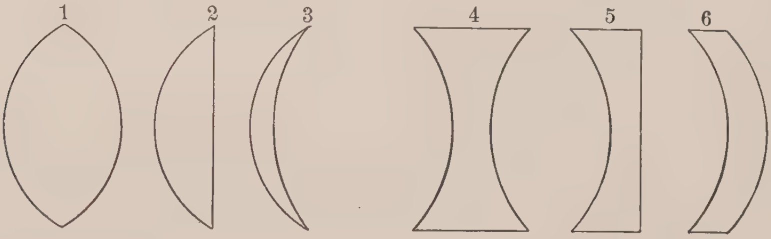
FIG. 52.



the ray of light passes from the glass in a direction parallel to its original direction. The ray *ab* had received no change in direction, but by virtue of its passing through the glass plate with parallel sides has changed its position. The eye of a person observing the ray at *d* would find no distortion of the ray, but would see its source at the point *c′* instead of *a*. This is what occurs when one looks obliquely through a window where the surfaces of the glass are supposed to be parallel.

If the planes which bound the transparent solid are not parallel but at an angle to each other we obtain an entirely different phenomenon. This is best illustrated by the prism. Fig. 52 shows the cross section of the prism *abc*. The ray of light *de* falls upon the surface *ab* of the prism at the point *e*, and is refracted toward the perpendicular *l′* erected at *e*, in such a manner that it takes the direction *ef*. At *f* it strikes the surface of the prism *bc*, and passing from the denser to the rarer medium it is refracted away from the perpendicular *l″* erected at *f*, in such a manner that it takes the direction *fg*. One sees at a glance that here the direction of the ray of light has been markedly altered. The various proportions of the angles of impact, refraction and exit, will be seen to bear the constant relation to each other which has just been stated. There are, however, bodies of glass so shaped that their refracting surfaces are not smooth but curved. These are the so-called lenses. There are various types of lenses, which are employed singly or in combination in the construction of the microscope. Of these there are six different varieties, which may be divided into two principal groups of three each. Those in the first group, illustrated as 1, 2, 3, in Fig. 53, are thicker at the center than at the periphery, and are the so-called convex lenses. No. 1 is bounded by two convex surfaces; hence is called a double convex lens; No. 2 is bounded by one plane and one convex surface, and is called plano-convex, and No. 3, a lens having one concave and one convex side, is the so-called concavo-convex lens. Those in the second group are thinner at the center than at the periphery. These are the so-called concave lenses. No. 4 is a double concave lens, No. 5 plano-concave, No. 6 a convexo-concave. Convex varieties are also designated as collective lenses, the concave varieties being known as dispersion lenses. If a bundle of parallel rays of light fall upon a double convex lens we have a means of analyzing the directions which the various rays of light would take by conceiving a lens to consist of an innumerable number of superimposed prisms. If we begin with a cube of glass, as shown in Fig. 54 and place upon it a prism and upon it a second and so on, each prism having a more acute angle and these prisms so numerous that at every point the direction of the surface would change, we can see that the result of these innumerable prisms would be represented by a curved surface on both sides, and that we would have a double convex lens.

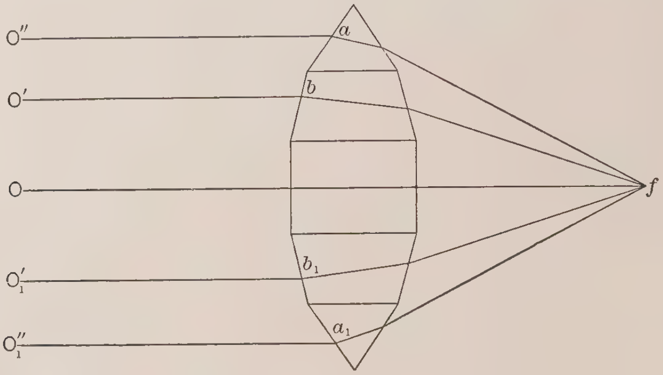
FIG. 53.



In order to understand the direction which parallel rays of light falling upon a lens would take, we may conceive of the lens being divided into simple prisms and select such rays of light as would justly fall upon their surfaces. This is illustrated in Fig. 54.

The ray of light *of*, falling upon the center of the lens, which at the point of entering and exit has parallel sides, would pass straight through the lens and traverse the point *f*; the rays of light *o′* and *o″*, falling upon the surfaces of the prisms at *b* and *b′* are refracted at these points toward the axis, traverse the prisms and passing through the opposite surfaces they are refracted away from the perpendicular and likewise fall upon the point *f*. The rays of light *o''* and *o''* fall upon the surfaces of the prisms at *a* and a_1, where the angles are greater than at *b* and b_1 and are refracted in the same manner, falling at the point *f*.

FIG. 54.



To determine this point we may conceive of the opposing convex surfaces as segments, or portions of the surfaces of the spheres having their centers at *m* and *m'* (Fig. 55). If we draw a line through the points *m* and *m'* it will pass exactly through the centers of the two surfaces, and represents what is known as the optical axis of the lens. A ray of light falling in the optical axis would pass directly through the lens, and would not be refracted. If, instead of the schematic lens composed of prisms, shown in Fig. 54, a double convex lens was substituted, as in Fig. 56, and we conceive of a bundle of parallel rays

FIG. 55.

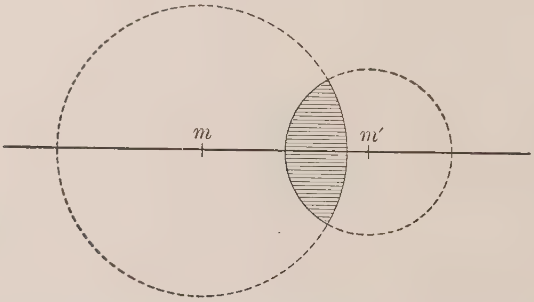
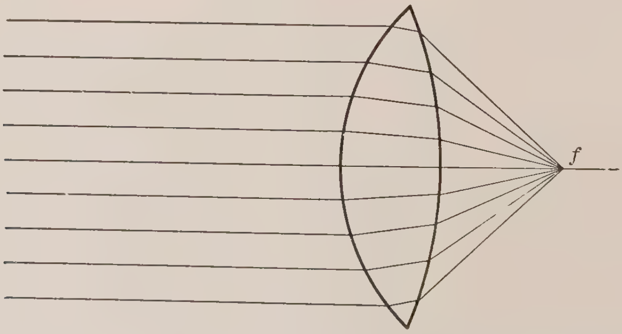
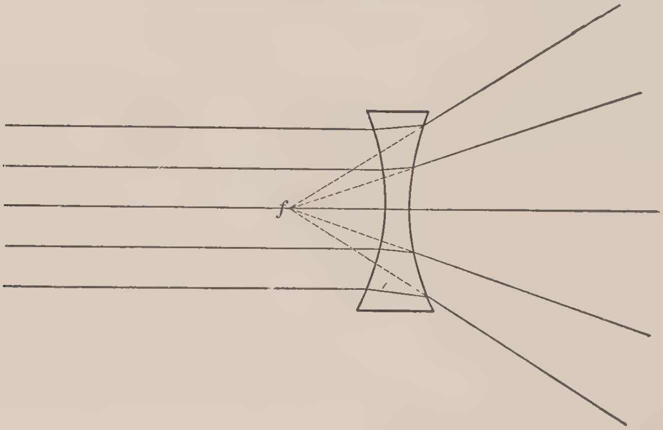


FIG. 56.



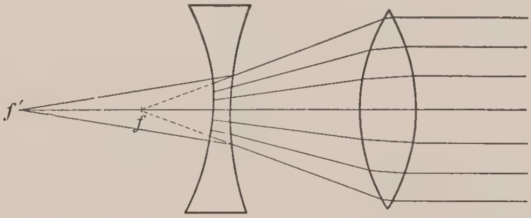
of light falling upon its surfaces, we will observe that they are all refracted toward the axis and meet in the point *f*. If we invert the relation, and conceive of the source of light placed at *f*, we will see that the rays of light radiating from it and falling upon the surface of the lens will be refracted toward the axis and will pass from the lens parallel to each other. The point *f* is the so-called focal point of the lens, and the distance from the center of the lens to the point *f* is called the focal length of the lens. Parallel rays falling upon the surface of a concave or double concave lens change their direction, being refracted away from the optical axis, and are dispersed. (Fig. 57.)

FIG. 57.



We see from this illustration that parallel rays of light passing through a concave lens can never meet; therefore, no real image can be produced. The theoretical image may be obtained by prolonging the radiating lines backward, when they will meet at the point *f*, which is the focal point of the double concave lens. The focal length is the distance between the center of the lens and the focal point. Likewise, converging rays of light having the direction of the lines meeting at *f*, falling upon the surface of the concave lens, would be projected parallel to each other. The action of placing a double convex and a double concave lens together may be seen in Fig. 58, in which case it is possible for the concave lens to project a real image, which does not fall at the point *f*, the focal point of the convex lens, but at the point *f'*. The position of the point *f'* is dependent upon the focal lengths of the convex and the concave lenses and their distances from one another, and can be determined by mathematical calculations with which we shall not concern ourselves. The utility of thus combining convex and concave lenses of various forms and focal lengths, producing variations in the point where the image falls, by altering the relative distance of the lenses and the possible results that may be obtained by using

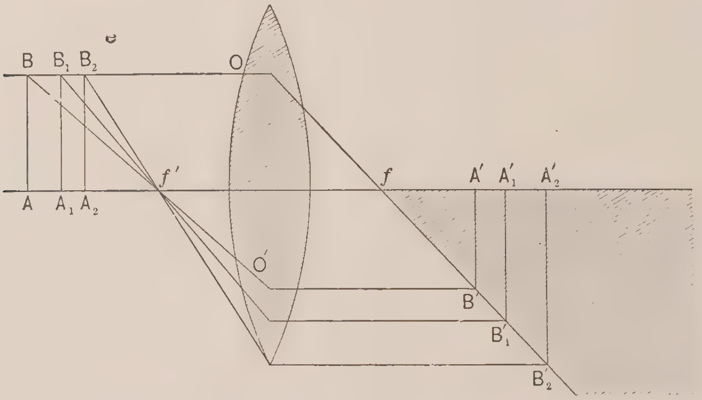
FIG. 58.



such combinations in constructing microscope objectives, may be readily appreciated.

So far we have only considered the direction taken by parallel rays of light in passing through a lens. We must now consider the direction which rays of light emanating from an object would take in such a passage. If the object is a line it is evident that the direction of two rays of light, one passing from each end, would be sufficient to determine the position of its projected image. If it is a solid body we must conceive of theoretical lines which include the principal points of the object and determine the image from these. Fig. 59 illustrates a double convex lens and the course which rays of light from an image AB would take in passing through the lens. First we must conceive that the lens has no thickness, which simplifies the determination of the direction of the light rays. *f* and *f′* are the two focal points of the lens and therefore the line *ff′* represents the optical axis. AB is the theoretical line from which the rays of light passing from its ends A and B through the lens are to have their direction determined. We see at once that as A lies in the optical axis the ray of light from A to A′ passes directly through the center of the lens and is not refracted. This point A is taken to simplify the demonstration. When we have determined the direction which the ray of light from B takes, we may readily determine any point between A and B. Let us first consider two rays of light passing from B, the first, BO, which may be taken parallel to the optical axis, and the second BO′, which passes through the focal point *f′* and must, therefore, pass out of the lens parallel to that axis. The point at which these projected rays cross is B′; therefore at this point, B′, must fall the image of the point B, and by erecting a perpendicular from the optical axis to the point B′, the image of line AB is shown to be the line A′B′. It will be seen from the diagram that the image of the line AB produced by a convex lens is a reduced inverted image. If the line AB be moved away from the lens the point B′ would move gradually toward *f*, in the direction of *f*B′, the image A′B′ becoming gradually smaller as the line AB is moved backward. At the point where the image AB becomes infinitely distant from the lens, the point B′ would fall at *f* and the image would become infinitely small. If, however, the line AB is in the position A′B′ at a distance in front of the lens equivalent to twice its focal length in that direction, the image would fall at A′′B′′. We may also see from the figure that the image is now exactly the same size as the object and lies at a point behind the lens at a distance equal to twice the focal length in that direction when focal lengths are equal. In other words the

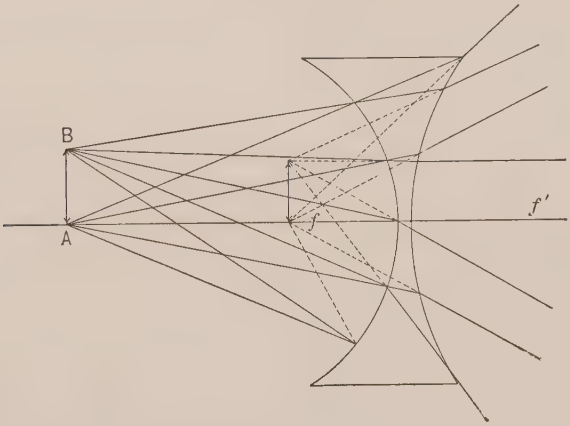
FIG. 59.



object is the same distance from the lens as the image and both are alike in size. If the line AB be moved toward the lens, for instance, to the position A_2B_2, the image will fall at $A_2'B_2'$, proportionately farther away from the lens, and when the projecting line AB falls at the focal point f' the image will be projected to infinity. We see also that all images projected by the lens fall within the angle $A_2'fB_2'$ and that the size of the image is dependent upon the distance of the object from the lens. If we attempt to analyze these facts we may deduce several rules. The real projected image from a double convex lens is always inverted and lies between the focal point and a point equal to twice the focal length of the lens when the object is placed at a point distant more than double the focal length from the front of the lens. If the object is placed at a point equal to twice that focal length, the image will be formed at a point behind the lens equal to twice the focal length in that direction and will have the same dimensions as the object. If the object lies at a point between the double and the real focal length before the lens, the object will be projected to a point beyond the double focal length behind the lens and the image will be magnified.

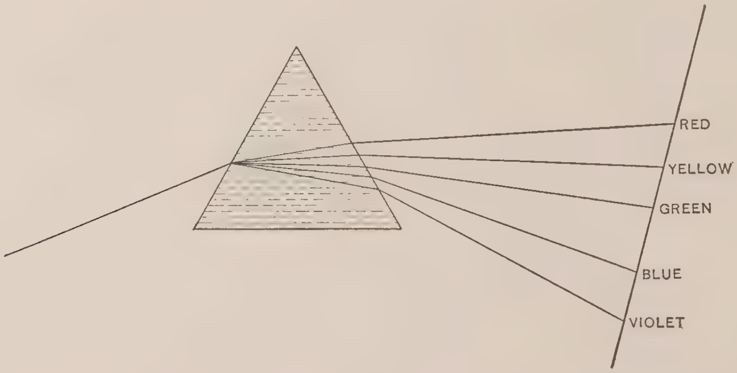
Dispersion lenses do not project a real image. The accompanying figure (Fig. 60) shows that the image when constructed by lengthening the projected lines backward will be seen at f and will be an upright instead of an inverted image.

Fig. 60.



With this brief statement of the optical qualities of lenses we may pass to the consideration of the second most important phenomenon produced by the passage of light through a transparent medium, that of dispersion. The various phenomena of refraction which we have just studied apply only to monochromatic light. We have observed that various kinds of light have distinctive amplitudes of waves. Upon the character and length of the wave depend most, if not all, of the characteristics of the light. Monochromatic light is that which is produced by vibrations of ether, all of which have the same wave-length and amplitude. Such light may be obtained when sodium salts are burned in the flame of a Bunsen burner. If before such a burning sodium flame be placed a screen having a narrow slit and a prism be placed between the slit and the white screen, a picture of the slit will be projected through the prism, the direction of the refracted rays being as indicated on a previous page. If in place of the sodium flame we allow sunlight to pass through the prism, we find instead of a single yellowish band a series of colors which form the so-called solar spectrum. This solar spectrum contains the colors red, orange, yellow, green, blue, indigo and violet (Fig. 61). This phenomenon is thus explained: The rate of transmission of light through a medium is dependent upon its wave-length; as the several colored lights composing sunlight have different wave-lengths, in passing through the prism each is refracted differently and falls upon a different point in the screen; the red, having the longest wave-length, being refracted the least; the violet the most, as it has the shortest wave-length. From this phenomenon is derived the statement that the violet rays are more refractible than the red. The order of arrangement of colors in the spectrum remains the same no matter what the character may be of the transparent substance composing the prism. From the foregoing it will be seen that the refractive index of a transparent medium varies with the kind of light employed. It is, therefore, necessary to establish a standard, and for this purpose we select light from a certain portion of the spectrum. A fact of the greatest importance in this connection is the presence within the solar spectrum of certain distinct dark lines which have a fixed position and are known as the lines of Fraunhofer, after the name of the scientist who first investigated them. A spectrum is only continuous or complete so long as the light which produces it radiates from a glowing solid or fluid. If the spectrum of glowing gases be taken, such as hydrogen, nitrogen, etc., or of the gases from vaporized solids, we find that only a portion of the spectrum is present, that is, the glowing gases furnish only certain colored lights of varying wave-lengths, inasmuch as the spectra of various gases differ extremely, some showing the presence of only one or two colored lights, others of many. If, however, the spectrum of a glowing solid or fluid be allowed to pass through a glowing gas certain portions of the spectrum remain black, that is, certain colored lights emanating from the glowing solid have failed to pass through the glowing gas. This phenomenon is known as the absorption of light, and these areas of absorption represent the position of the colored lights which emanate from the glowing gas. The lines of Fraunhofer in the solar spectrum are produced by the absorption of certain portions of the colored lights composing sunlight in its passage through the atmosphere of the earth. The heaviest of these lines are for convenience designated by letters of the alphabet from A to H and occupy certain definite positions in the spectrum which can be seen in Fig. 62. Their relation to the colored lights which compose the spectrum has been accurately determined, as well as the wave-lengths of the light whose absorption has produced them. As we have stated, it is of great importance in determining the refractive index of a transparent medium to use a monochromatic light and to be able to estab-

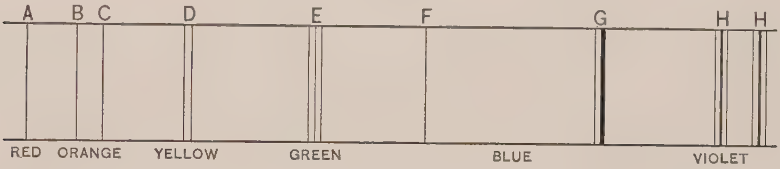
Fig. 61.



lish its position in the spectrum with certainty. For this purpose yellow light reaching the spectrum at the D line is taken, and in works on optics, this is always expressed by the formula $n\text{D} = 1.43$, which means that the refractive index of the substance, which is always expressed by the letter n, is 1.43, when tested with yellow light taken from the position of the line D in the solar spectrum.

By the term glass are understood various transparent substances of different chemical composition. These contain silicates and alkaline metals, especially calcium, potassium and sodium combinations, lead and borax. Glass is made by melting these various substances together, after which they cool into a homogeneous transparent mass, which does not crystallize. By varying its constituents glass of varying optical qualities may be obtained, and it has been largely due to the development of technique in glass manufacture and the production of greater varieties of optical glass that the recent marked advances in objective construction have been made. These varieties are divided into two general classes according to their optical qualities, those with a high refractive index and relatively high dispersion[1] being grouped together and classed under the general term of flint glass. Chemically flint glass is a calcium plumbic silicate combination. The second class is characterized by a low refractive index and relatively small dispersion, and is known as crown glass, being a chemical combination of calcium and potassium silicates. In reality this division is very arbitrary, especially in the more modern varieties of glass, where all grades between the two have been produced, as well as some grades which possess the high refractive index of flint glass and the low dispersion of crown glass. It is especially due to the increase in glasses possessing these two characteristics in such variety, that the beautiful correction of the modern microscope objectives has become possible. In order to understand some of the difficulties which have been overcome, let us consider the characteristics of the so-called achromatic objectives. We have seen that a lens may be conceived as composed of an infinite number of prisms; therefore, a ray of light passing through a lens is exposed to the same action as light passing through a prism. We have seen that a ray of light passing through a prism is refracted and dispersed by reason of the differing refractability of the colored rays composing white light. The phenomenon of dispersion occurs when a ray of white light falls upon the surface of a lens. Once separated into bundles of different colored light, each color is refracted differently and converges at a different point, the rays of light of the same color falling together. In order to clearly understand this let us proceed with monochromatic light. If parallel rays of blue light be allowed to fall upon the surface of a convex lens, they would converge at the point f (Fig. 63) which is the focal point of the lens for blue light. If at the same time we allow a similar bundle of parallel rays of red light to pass through the lens they will not be refracted as much as the blue light, and will fall at the point f'.

Fig. 62.



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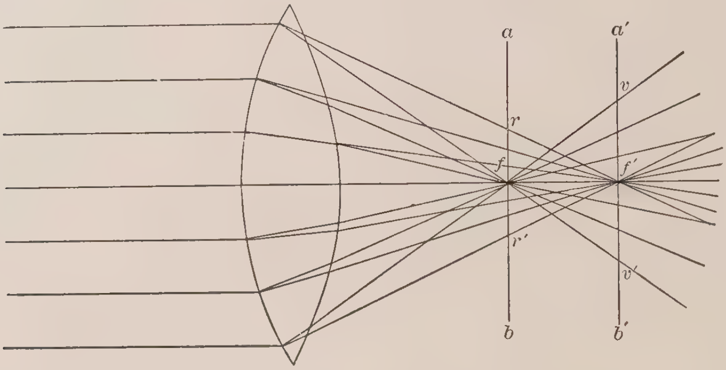
[1] By low or high dispersion is meant the relative difference in refraction to which the colored lights of the spectrum are exposed. Upon this is dependent the width of various portions of the spectrum.

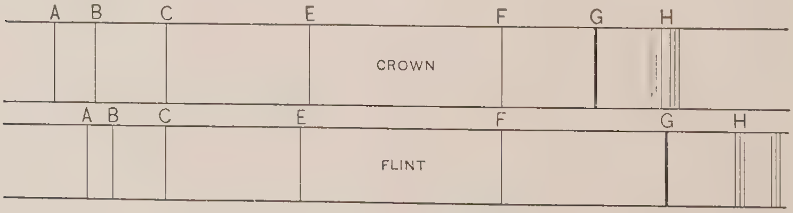
If then we place a screen *ab* at the first point, we will have a brilliant blue spot at *f*, surrounded by a halo of red between the points *r* and *r'*, and if the screen be moved to the point *f'* we would have a central bright spot of red, surrounded by a halo of blue lying between the points *v* and *v'*. This illustrates in a simple manner exactly what occurs when white light passes through a single lens. The violet rays being refracted the most have their focal point nearest to the lens, the red, being refracted the least, have their focal point at the greatest distance from the lens and the various colors of the spectrum occupy focal points intermediate between these in the order of their refraction. To correct this dispersion of white light there is a means at our disposal. The simplest demonstration of this means is shown in the construction of the so-called achromatic lenses. (See diagram C, Pl. XXXVIII.)

Fig. 63.



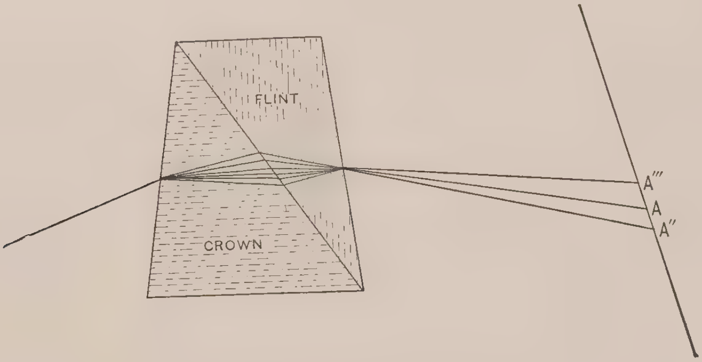
In order to understand what occurs when a suitable prism of crown glass is placed upon a prism of flint glass, or what occurs when lenses composed of crown and flint glass are placed together, it is well for us to compare the spectra of the two glasses. These are shown in Fig. 64. We see that the two spectra between the lines C and F have the same width, that is, the colored lights which fall between C and F orange, yellow and green, are refracted equally. Therefore, the dispersions of the spectra between these points for the two kinds of glass are equal. When we examine the spectrum between A and C we find the distance between A and C is wider for crown than the distance between these lines in the spectrum for flint glass. At the other end of the spectrum we see the reverse condition, the distance between F and H being less in the crown than in flint glass. Therefore, the length of the spectrum in crown glass is maintained at the expense of the more refractable colors, blue and violet; whereas, in flint glass the reverse occurs, the length of the spectrum being maintained at the expense of the colors between A and C, the less easily refracted

Fig. 64.



red and orange. We may see from these spectra that if prisms of flint and crown glass so constructed that the spectrum of each prism between C and F has the same length, are placed together (Fig. 65), the colors lying between the lines C and F would be subject to an equal amount of dispersion, and therefore, these colors being dispersed by the first prism would likewise be collected by the second, and would issue from the prism as white light. The relation of the colors at the ends of the spectrum show that they would not compensate for each other. The ray of white light would fall at the point A on the screen, but the refractable violet rays, being strongly refracted by the flint glass, would fall at a point above the white light, for instance at the point A‴, whereas, the less refracted red rays would fall at a lower point A″, being insufficiently refracted by the flint-glass prism. As a result, beside the ray of white light, the violet and the red rays of the spectrum would be visible. Persistence of these two colors of the spectrum produce the so-called secondary spectrum, which is always present in the older form of achromatic objectives. The term achromatic is, therefore, a misnomer, as all the colors of the spectrum have not been perfectly corrected. Without wearying the reader with detail, it may be stated that by the advances made in the manufacture of glass it has been possible to produce prisms or lenses having optical qualities which exactly compensate for each other. A combination of this kind is produced when two suitably constructed prisms or lenses, one of barium phosphate crown glass and the other of borate flint glass, are placed together.

Fig. 65.



The application of this principle to the construction of microscope objectives was first made by Abbe, who formulated a series of objectives which were produced by Zeiss under the technical term of "apochromatic objectives." In these objectives the colors of the secondary spectrum are eliminated from the visual field. Many of the new forms of glass have, however, one serious defect in not being durable, and these have a tendency to deteriorate, especially when exposed to variations of temperature and moisture. This is usually shown by the oxidation of the lens surface, destroying its polish and brilliancy, while occasionally a lens becomes clouded throughout its entire substance. This has been one of the greatest drawbacks to the use of apochromatic objectives, and has been but recently overcome. The recent lenses of Zeiss are entirely durable.

Qualities which are desirable in a microscope objective are: high aperture, flatness of field, correction of spherical aberration, correction of chromatic aberration, including secondary spectrum (freedom from color), and durability.

APERTURE.

Upon the aperture of the microscope objective are dependent various qualities: First, definition. This is primarily influenced by the correction of the objective. If an objective is properly constructed and its correction is as perfect as that of another objective of similar focus or resolving powers, the definitions of the two are in direct proportion to their apertures. This is expressed in degrees of the so-called angle of aperture, which is the angle made by the most peripheral or diverging light rays from the axial point of an object that can enter an objective and help to form the image. The defining power of an objective is, therefore, directly dependent upon the number of the rays of light which can be collected by the front lens of its systems and which can take part in the production of the final image. It will be seen that the angle of aperture decides the shape of the front lens. A moment's consideration will show us that in order to obtain the highest aperture possible the front lens must be a hemisphere. In fact, in the objectives of high power, as, for example, oil immersion objectives, this form is employed, but in low power objectives it is not practicable to employ it.

Thus far we have considered the angle of aperture of objectives for rays of light passing from the object through the air into the front lens. The direction which these rays of light take is dependent upon the refractive index of air. If a transparent medium of greater refractive index than air be placed between the front lens and the object, more of these rays will be refracted toward the axis, so that a greater number will fall upon the surface of the front lens. More rays will reach that lens if transmitted through water than if through air; a greater number still if the transparent medium be a homogeneous immersion oil, and objectives are especially constructed for use with each of these different media. A simple statement of their angle of aperture, therefore, will not accurately express the relation of results obtained in using respectively the dry, the water immersion and the homogeneous immersion objectives, for both the medium of transmission and the angle of aperture must be taken into account in considering the rays of light that actually pass from the back combination of each of these objectives to form the real image. To express this relation Abbe introduced the term "Numerical Aperture," devising a formula which should cover each of these considerations—

$$n \sin u = NA$$

in which NA is the numerical aperture; n the index of refraction of the transparent medium, which is multiplied by the sine of u, representing one-half the angle of aperture.

As the outer surface of the front lens of an objective must always be separated from the object by a definite distance, however small (owing to the presence of the cover-glass), the angle of aperture of any lens can never be as great as 180 degrees, and as half of that angle can never be greater than 90 degrees the numerical aperture of a dry lens must always remain less than one. A dry lens cannot have a numerical aperture greater than .95. The refractive index of water is 1.33, that of cedar-wood oil used for oil-immersion objectives is usually of the same refractive index as glass, 1.52. However, in practice it is not feasible to employ water immersions with a greater numerical aperture than 1.20 or oil immersions with a greater numerical aperture than 1.40. There are various means by which the aperture of a lens may be increased, one or two of which deserve special mention. The refractive index of ordinary glass being relatively 1.54 it is useless to employ an immersion fluid of higher index unless the glass which is to receive the rays has as high or a higher refractive index. For this reason glass of high refractive index is used in the front lenses of microscope objectives. At various times attempts have been made to increase the aperture by using substances of higher refractive index, such as the diamond, which has a refractive index of 2.4. No lenses employing diamond as the material for the front lens have ever been successful, owing to the great technical difficulties in preparing them, which renders their grinding and polishing a practical impossibility, although frequent attempts have been made to accomplish this. A second method of increasing the numerical aperture of a high-power objective is that of placing a negative lens over the positive front lens, thus causing the combination to approach nearer to the object. This is a doubtful expedient, as it shortens the so-called working distance of the objective. A technical limitation placed upon actually obtaining high aperture in high-power objectives is the extent of periphery in the front lens, which is covered by the mount in which it is supported. We have observed that in oil-immersion objectives a hemispherical lens is used. In order to hold this in place a narrow rim of metal is placed against the margin of the lens. In proportion as this rim of metal is stout or thin so is the diameter of the available opening of the lens diminished. As the solidity with which the front lens is held in place depends upon this mounting, it is a matter of great importance for the durability of the objective. In the apochromatic oil-immersions made by Zeiss those which have a numerical aperture of 1.40 are so constructed that the front lens is held in place by a very narrow margin, the lens being pressed into position from behind and held in place by cement. It is technically impossible to construct an oil-immersion objective in which the front lens is held securely in place that will have a higher numerical aperture than 1.35, and it is well to bear this in mind in their selection. The small gain in definition between a lens with a numerical aperture of 1.35 and one in which that aperture is 1.40 is practically of no moment. Beside this, an oil-immersion objective in which the numerical aperture is 1.40, requires the greatest caution in its use and is very liable to injury. The authors have known several instances where the front lenses of immersion objectives of high aperture have become loosened simply by the expansion of the mount produced by using them in connection with a warm stage. The difference in brilliancy of the images between those given by oil-immersion objectives of 1.25 *NA* and of 1.35 *NA* is sufficiently great to be of importance in practical work. The difference in cost between objectives of low and those of high aperture is of necessity very great.

FLATNESS OF FIELD.

Flatness of field in the ordinary achromatic systems is dependent upon the construction of the objective. In the apochromatic systems the ocular, likewise, aids in producing a flat field. The curvature of a field in any system may be lessened by diminishing the aperture of the objective. For this reason objectives of low aperture usually show less curvature of the visual field than those of high aperture. For photomicrography only systems possessing a relatively flat field can be employed.

Lenses which owe their flatness of field to diaphragming suffer such a marked reduction in definition that they are unsuitable for finer photomicrographic work. In this connection the lenses of Winkel possess the quality of high aperture and extreme flatness of field. The higher numbers of the Zeiss apochromatic objectives possess these qualities to an almost equal degree. The lower numbers, especially the 16 mm. lens of Zeiss, have a marked curvature of the visual field, which diminishes markedly their utility for photomicrography.

SPHERICAL ABERRATION.

By spherical aberration we mean a defect in the optical performance of a lens the result of lack of coincidence in the focal point of parallel rays entering the lens at the axis with the focal point of similar rays entering the lens at the periphery. This phenomenon is best understood by considering the accompanying diagram (Fig. 66). If we conceive that four parallel rays of light, a, b, a', b', of which a and a' strike the lens at the periphery and b and b' near the axis, we find that b and b' meet at the focal point of the lens f', a and a', however, striking the lens at O and O''', meet at the point f, which lies nearer the lens than f'. The distance $O'S$, $O''S$ being the distance from the axis of b and b', is but a small fraction of the focal length of the lens; therefore, these two rays of light meet at the focal point. a and a', however, having the distances OS' and $O'''S'$ from the axis, which is a considerable fraction of the focal length of the lens, do not fall at this point. All of the intermediary rays between aO and bO' and $b'O''$, $a'O'''$ will have their focal points between f and f'. This phenomenon is called spherical aberration and the distance between f and f' represents the amount of spherical aberration in the lens. If the parallel rays of light which fall upon the face of the lens are composed of monochromatic light and there be but the four rays of light indicated, then if we place a ground-glass at the point f', we may see a bright illuminated spot with a circular halo about it, produced by the rays of light which have met at f. If we place the ground-glass at f, we again see a bright central spot with a somewhat smaller circle of light surrounding it. In the ordinary bi-convex lens the parallel rays of light having their focal point upon the axis fall very near together, and it is only when we depart from the axis that we find the phenomenon of spherical aberration becoming apparent. As we proceed from the axis we find the phenomenon increasing with great rapidity. Therefore by cutting off the peripheral rays from a lens, we may reduce the amount of spherical aberration. We see, likewise, that the rays of light which enter the lens at the periphery produce greater spherical aberration than those entering at the center; therefore, if we cut out the central rays and use only those at the periphery, we would find the phenomenon increased. We see that in a convex lens the peripheral rays have a shorter focal length than the central rays. This is called positive spherical aberration. In a biconcave lens the condition is reversed, the peripheral rays having a longer focal length than the central. This is called negative spherical aberration, and in a plano-convex lens the spherical aberration is less than in other forms of lenses.

FIG. 66.

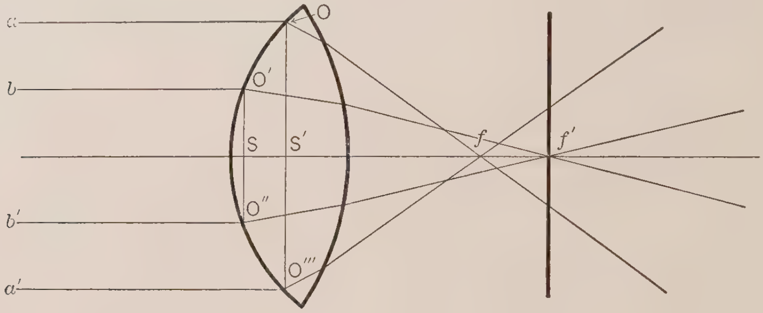
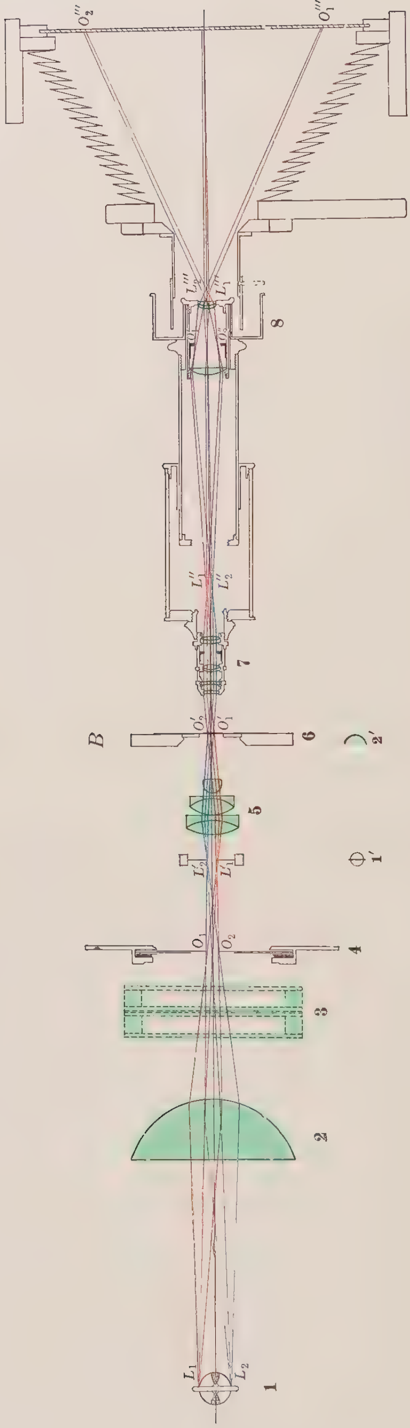
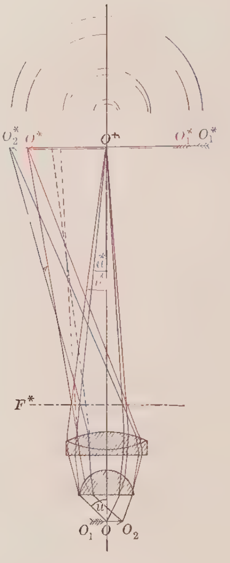
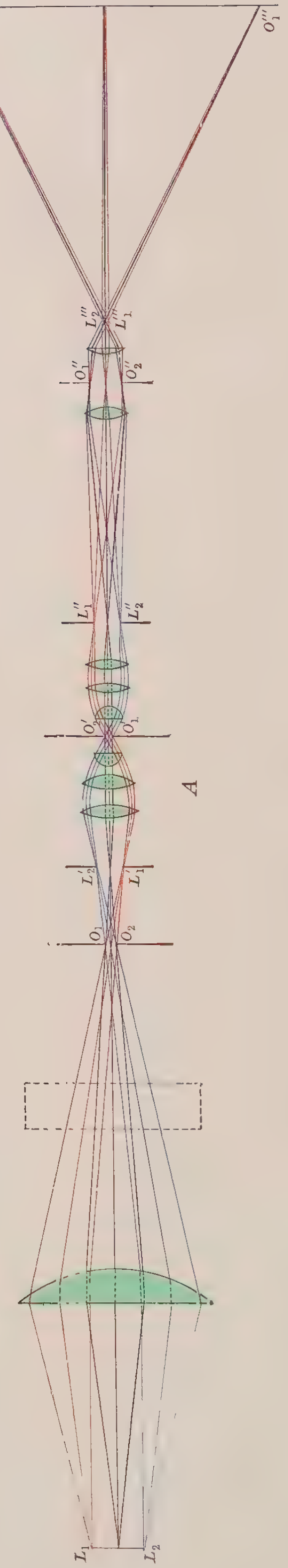


PLATE XXXVIII.







There are various means of correcting spherical aberration, one of which is so constructing the lens that by its form the spherical aberration is reduced to a minimum. In microscope construction the spherical aberration is corrected by compensation, a lens having positive spherical aberration being combined with a lens having negative spherical aberration, the two equalling each other. A further phenomenon which has to be corrected in microscope objectives is produced by the fact that rays of light from different portions of the spectrum produce different amounts of spherical aberration and is corrected by using glass of suitable refractive index and dispersion. In the ordinary achromatic objective the spherical aberration is only well corrected at the axis, and there remains in almost all objectives of this type a certain amount of spherical aberration outside of the axis. In the apochromatic objectives the spherical aberration even of the peripheral rays is practically eliminated.

CHROMATIC ABERRATION.

As we have seen, a suitably constructed prism of crown and flint glass will correct the dispersion of the rays of light between C and F of the spectrum. This leaves the rays of light near the ends of the spectrum uncorrected, and we find in all achromatic objectives a halo of color composed of red and blue about the edges of all colorless objects. For ordinary ocular observation this defect in the achromatic objective produces no serious disturbance; as the yellow and green rays are the most brilliant the less brilliant blue and red do not materially disturb the image. The lack of uniform convergence of the red and blue rays in the achromatic objectives produces another condition which is known as chromatic difference in magnification. This is due to the fact that the uncorrected red and blue rays, owing to their difference in wave-length, possess different focal lengths, and we have the red rays meeting at a point nearer the lens than the blue. Therefore, an image of an object produced by the red rays is larger than the image produced by the blue rays without altering the distance between the object and the lens. The accompanying diagram (Fig. C, Plate XXXVIII), after Czapski, illustrates this defect in the achromatic objective. It will be seen that the central rays emanating from O in the object meet in the axis at O′, and that here the red and blue rays nearly coincide. As we proceed from the axis toward the periphery of the visual field we find that the rays of light emanating from O″, being dispersed upon the front surface of the front lens, take a different course through the lens, the red rays being refracted the least and meeting at O″; whereas, the blue, the more refractable, meet at a point O″ which is above and without O″ of the red rays. Therefore, at the axis the chromatic difference of magnification is not as great as at the periphery. In the achromatic objective this difference between the chromatic aberration at the axis and at the periphery is, in great part, corrected by the use of a compensation ocular, and the error as it appears in the achromatic objective is practically overcome. In short, the principal difference between the chromatic correction of an achromatic and an apochromatic objective is that in the achromatic objective only the rays of light between C and F meet at the focal point, the red having its focal point below the junction of the blue and green, the blue having its focal point above, whereas, in the apochromatic objective all of the colors of the spectrum, not only at the axis, but at the periphery, unite at one focal point.

In photomicrography this complete elimination of chromatic difference of magnification in the apochromatic objective is of the greatest importance, and in tri-color photography, as will be seen later, absolutely essential.

DURABILITY.

Apochromatic objectives, owing to the nature of certain forms of glass which were employed in their construction, have obtained the reputation of lacking in durability. In the early objectives made by Zeiss this was unquestionably the case, but owing to experience obtained in the manufacture of these objectives and the introduction of new and durable forms of glass, this objection to the modern apochromatic objectives, at least to those of Winkel and Zeiss, as they are manufactured to-day, becomes obsolete. In the construction of the Winkel objectives this point was kept in view in the selection of the glass employed. The apochromatic objectives of Zeiss, as constructed to-day, are likewise perfectly durable. Objectives of this character require careful handling and should not be subjected to unnecessary changes of temperature or moisture.

PHOTOMICROGRAPHIC TECHNIQUE.

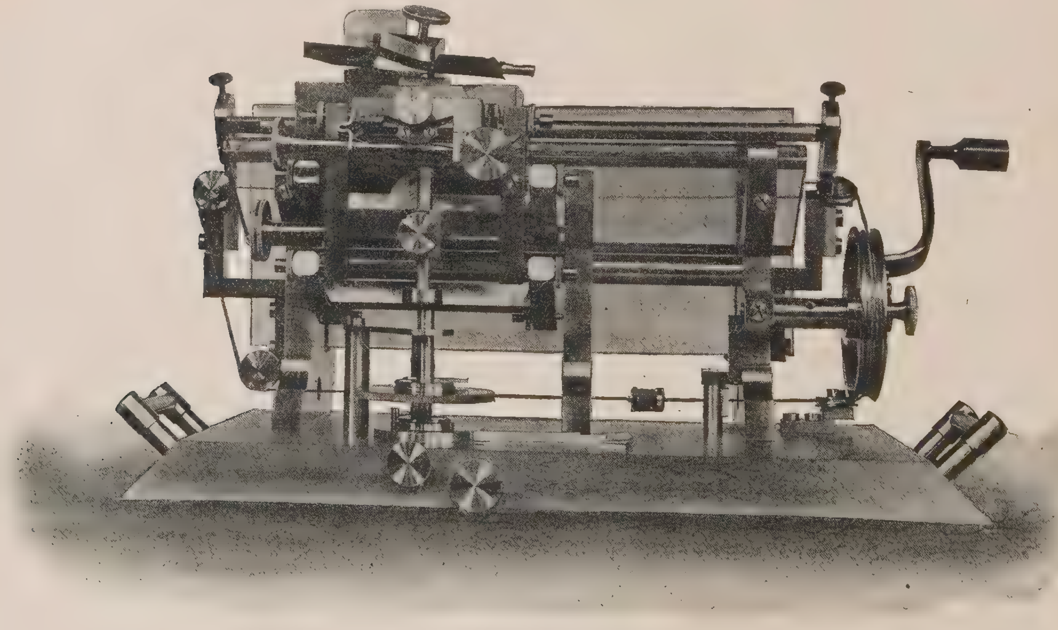
Equipment Required.

The marked improvement in apparatus and technique during the past few years has so markedly improved the quality of photomicrographs that their general introduction as a means of illustration and demonstration may be confidently expected. Good photomicrographs may be made with very simple apparatus, but the possession of specially constructed apparatus lightens the task and insures greater uniformity and certainty in the result. First of all, a good microtome is essential.

Microtomes.

There are many good microtomes. The very best are those made by Becker in Göttingen, and the Blake-Minot ribbon microtome made in Boston. As a universal instrument the sliding microtome of Becker (shown in Fig. 67) is

FIG. 67.



Becker's sliding microtome.

probably the best. With this microtome paraffine and celloidin may be cut equally well. The microtome slides are made of glass and the carriage rests upon bone feet, thus reducing the wear to a minimum. The preparation is supported on parallel arms and is raised and lowered for purposes of adjustment by means of a rack and pinion. During cutting the apparatus is adjusted by a perpendicular screw which is fed by an automatic mechanism. It has a useful clamp adjustable in two directions by screws, and a convenient mechanical motion with a handle to the right of the instrument which insures great accuracy in cutting fine sections. With this microtome large paraffine sections may be cut as fine as 4 microns, smaller ones, under favorable conditions, to 1 or 2 microns. The thinness to which celloidin sections may be cut depends so largely upon the success of the imbedding that no definite rule can be given. They may, however, under favorable conditions, be cut as thin as 4 microns.

The Blake-Minot microtome is an improvement upon the original ribbon microtome of Minot, and for cutting paraffine sections, especially in series, this microtome is unexcelled. For those who can possess but one instrument the sliding microtome of Becker is to be preferred.

Objectives.

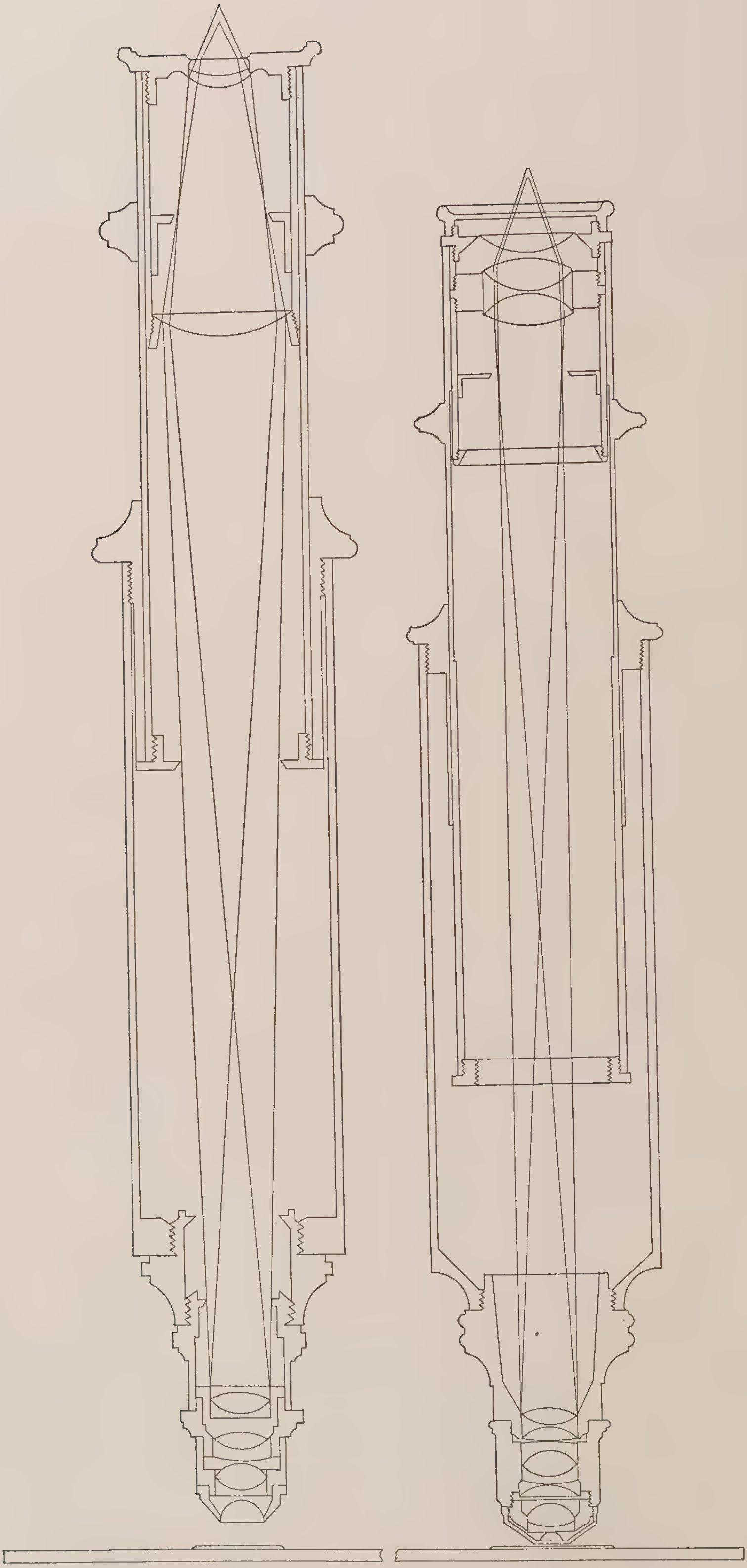
For photomicrography the apochromatic lenses are far superior to the ordinary achromatic objectives. Of lenses of this type practically those only of Winkel and Zeiss may be considered. Between the high-power objectives of these two makers there is no choice. In middle and lower powers the objectives of Winkel possess a superior flatness of field. All objectives higher than the 8 mm. of Zeiss are suitable for photomicrography. The 16 mm. of Zeiss has so marked a curvature of field as to render it useless for photography. The 10½, 14, 18, 24 and 32 mm. lenses of Winkel, with their respective oculars are a marked advance over previously constructed types of similar focal length. The complicated structure of the apochromatic type is shown in Fig. 68, which represents a 7½-mm. Winkel objective, with a No. 3 compensating ocular (Fig. 68, 1) and a Zeiss 3-mm. (1.40 N. A.) with a No. 12 compensating ocular (Fig. 68, 2). The tube length for the Winkel system is 182 mm. The compensation ocular shown (Fig. 68, 1) is of the negative type; that is, the focal point of the ocular is between the collective lens and the magnifying lens. The Zeiss ocular in this case is a positive ocular, having the focal plane of the ocular below the collecting lens. The tube length in the Zeiss system is 162 mm.

Lenses for Low-Power Photography.

In making low-power photographs of large sections for purposes of orientation and for the demonstration of the general relation of the various structures to each other a series of new objectives constructed after the type of the photographic lens may profitably replace the so-called projection systems which have been in vogue. The best lenses of this type are the micro-collinear objectives made by Voigtländer & Sohn and the Spencer Lens Co., or the planar lenses of Zeiss. The low-power micrographs in this book were made with the micro-collinear lenses. The correction of the rays outside the optical axis in these lenses is considerably better than in the planar objectives. The definition of the two types is about equal. A similar series of lenses constructed by Leitz are not to be recommended, as they do not possess sufficient aperture, their definition being greatly inferior to either of the above-mentioned series.

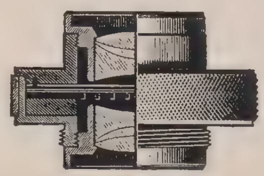
The micro-collinear objective is constructed after the well-known type of the collinear objective of Voigtländer & Sohn. The planar objectives of Zeiss are

Fig. 68.


1 2

constructed after the type of the well-known planar objectives of that firm. Both of these systems are mounted after the form of photographic objectives, and may be used either upon the front board of the camera or the smaller numbers may be screwed upon the tube of the microscope stand. These lenses are not employed with oculars, each being a complete system in itself. Fig. 69 shows a Spencer micro-collinear objective in cross section, from which it may be seen that the objective consists of two systems of three lenses each.

FIG. 69.



Micro-collinear 60 mm. with iris and society screw (full size).

The first is a bi-convex glass of low dispersion and high refractive index, $n = 1.613$, $r = 57.0$. The second is a meniscus collective lens of low refractive index and high dispersion, $n = 1.53$, $r = 43$. The objectives cover an angle of 65°, and are corrected anastigmatically within an angle of 50°, which correction coincides with the focal plane, so that the entire angle of 50° may be utilized with the full opening of the lens. These objectives are made in a series of different focal lengths beginning with 90 mm., 60 mm., 40 mm., and ending with 24 mm. For low-power photography they will be found to give much better results than microscope objectives of the same linear magnification. The principal advance is due to the great increase in the angle by means of which we are able to include a much greater area in the object with a lens having a much shorter focal length, and hence greater defining power than is possible with objectives constructed after the type of the microscope objective. For this reason these objectives should properly displace all microscope lenses having a focal length greater than 32 mm. The micro-collinear objectives have a relative opening of $F = 5.4$. In practice it will usually be found desirable to stop them down to $F = 6$.

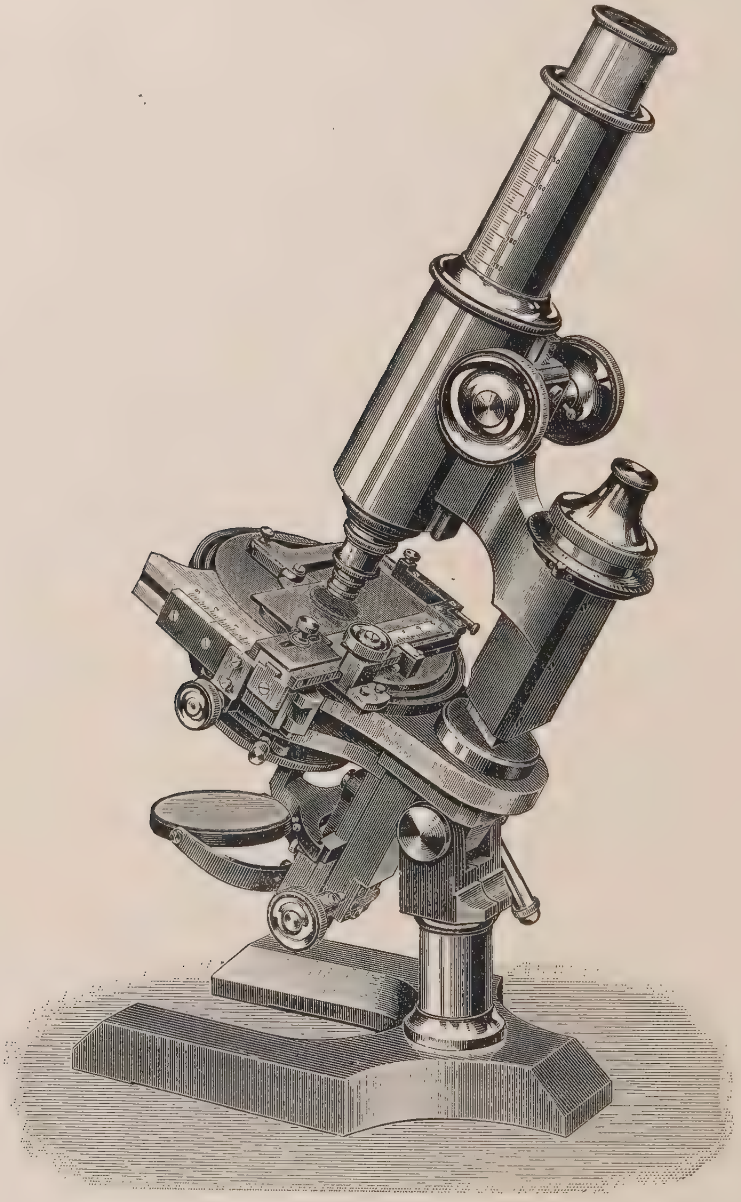
Microscope stands intended for photomicrography require some modifications in construction from the ordinary microscope stand. They must be supplied with a good mechanical stage, a large tube, a very sensitive fine adjustment, a centering, and in some cases an achromatic condenser. The stand manufactured by Winkel (shown in Fig. 70), and especially the new photographic stand of the Spencer Lens Co., fulfill all of these conditions. Either of these stands will render excellent service, and where they are not at hand an ordinary microscope stand with a good fine adjustment may be made to produce excellent results.

PRACTICAL MANIPULATION.

Description of Apparatus.

The first photographs for this work were made by means of a large Zeiss photographic apparatus belonging to the pathological institution in Göttingen. Shortly after employing this apparatus it was found that there were many points in connection with its arrangement and construction which could be simplified and that the entire construction of the apparatus did not offer the greatest possible stability, while by its construction it was very difficult to keep it perfectly aligned. As a result we were inclined to try the large apparatus of Winkel, which was constructed much after the pattern of Zeiss, but offered greater solidity, and in some ways more convenient construction. Among others, the table which supported the camera was so constructed that the rails upon which the camera traveled were made of one casting, so that the camera supports rested much more firmly upon this rail. It still possessed the great disadvantage in the minds of the authors that the entire apparatus was divided into two separate pieces; one supporting the source of illumination and optical appliances (the so-called illuminant bench) and the microscope being supported by a heavy iron table; the second division of the apparatus consisting of an iron support on which rested the camera. The centering of the apparatus was accomplished by centering the pieces upon the illuminating bench with the microscope and then placing the camera behind the microscope and bringing it into line with the optical axis of the first. This arrangement had the great disadvantage that any jar or accidental disturbance of the camera, or, in fact,

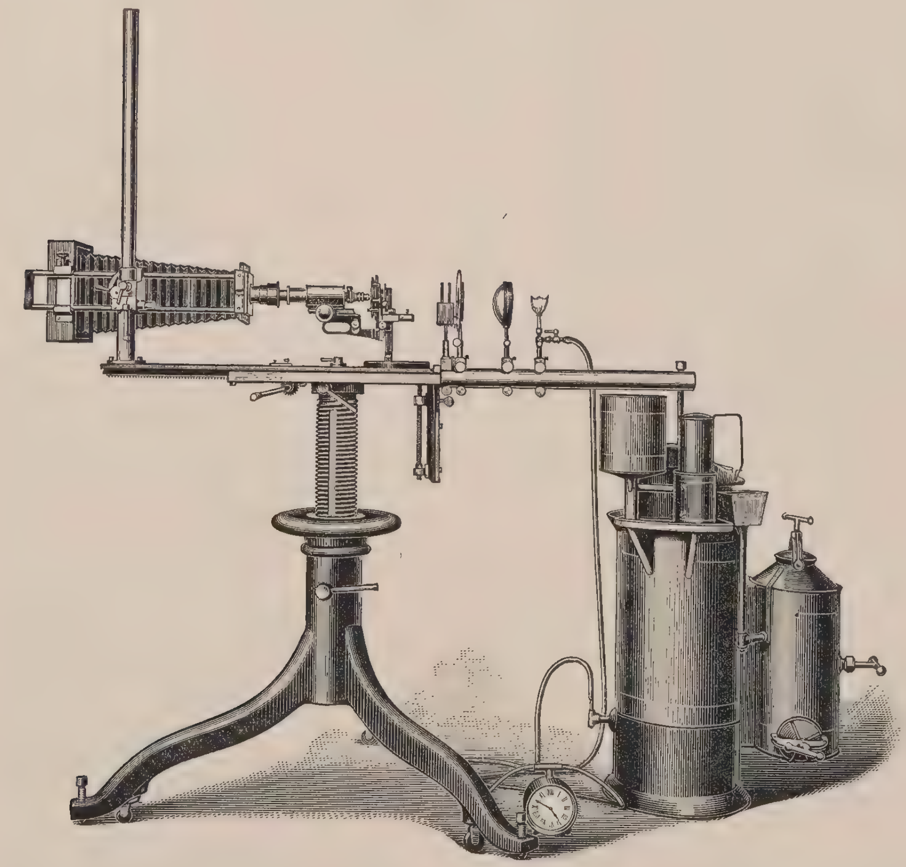
Fig. 70.



Large stand arranged for photomicrography. (Winkel.)

the effect of changes of moisture or temperature upon the wooden floor rendered necessary the recentering of the apparatus. In the Zeiss apparatus (at least the instrument at the Pathological Institute) this was somewhat difficult of accomplishment, but in the Winkel apparatus the feet of both tables were perforated by screws by means of which the tables could be leveled, raised or lowered. Even with this improvement (which was first applied by Herr Winkel) it was found that very slight jarring of the instrument could destroy the alignment. It, however, occurred to us that a new instrument could be devised which could offer all, if not more than all, the advantages of the cumbersome Zeiss apparatus, as well as that of Winkel of the older type, and which should possess the quality of resting upon but one support, thus having the advantage that simple jarring of the apparatus would not necessarily disturb the alignment of the various optical parts. To accomplish this, one of us (Gaylord) consulted with Herr Carl Winkel, of the firm of R. Winkel, and an apparatus was devised, the first form of which, although somewhat crude, was exhibited at the Naturforscher-vorsammlung, in Braunschweig, in 1897, and later a still more advanced development of the model was published in the *Zeitsch. f. wissent. Mik.*, Dr. Behrens, Bd. XIV. Since this publication the apparatus has been developed to the complete form which was decided upon in

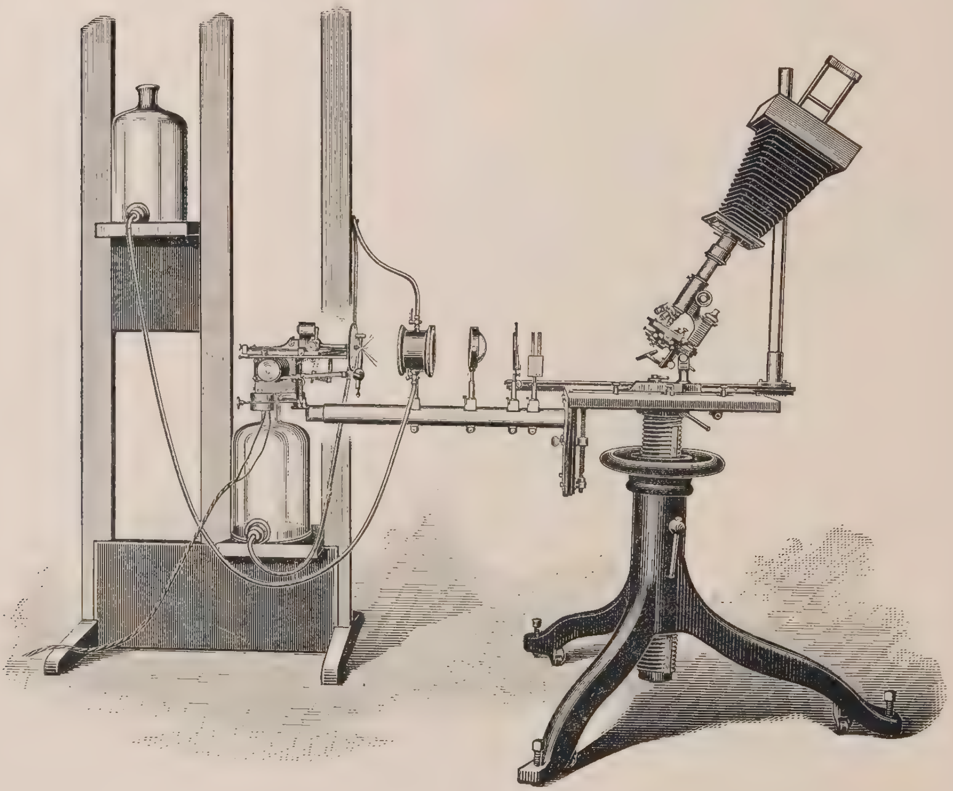
FIG. 71.



Photomicrographic apparatus in horizontal position. Arranged with acetylene gas as illuminant and clock for cutting off the gas at the completion of exposure.

the beginning, and about one-third of the photographs have been made with it. It has proven in every way to meet the most severe tests that could be placed upon it, and, by employment of the new Winkel fluorite system of objectives, has met every requirement. As was stated in the description published in Dr. Behren's *Zeitschrift*, the construction of the Fluorite system lenses is such that the oculars from 1 to 3 may be satisfactorily employed for photomicrography, and by this means the unduly long and cumbersome bellows of the larger forms of apparatus in vogue may be dispensed with. The apparatus consists of a flat iron plate upon which is placed the microscope, in either horizontal, inclined or vertical position as may be desired. The camera is supported by an upright resting upon a sliding plate, which is attached by a slotted groove to the base plate, which is so arranged that it may be drawn backward or pushed forward to the position desired. This is clamped fast by a winged screw, I. Upon the front of the base plate is cast an angle plate, upon which a slide plate may be raised and lowered by means of a screw (Fig. 74). From the slide projects a heavy metal tube, upon which are placed the parts of the optical illuminating apparatus and source of light. This tube and the appliances upon it form the illuminating bench. The appliances upon the illuminating bench are a collecting lens, a large iris diaphragm, a support for one or two ray filters and the source of illumination, which may be either limelight or acetylene gas, Welsbach burner or electric arc light. These appliances are arranged so that they may be raised or lowered, and by a special device moved transversely for

Fig. 72.

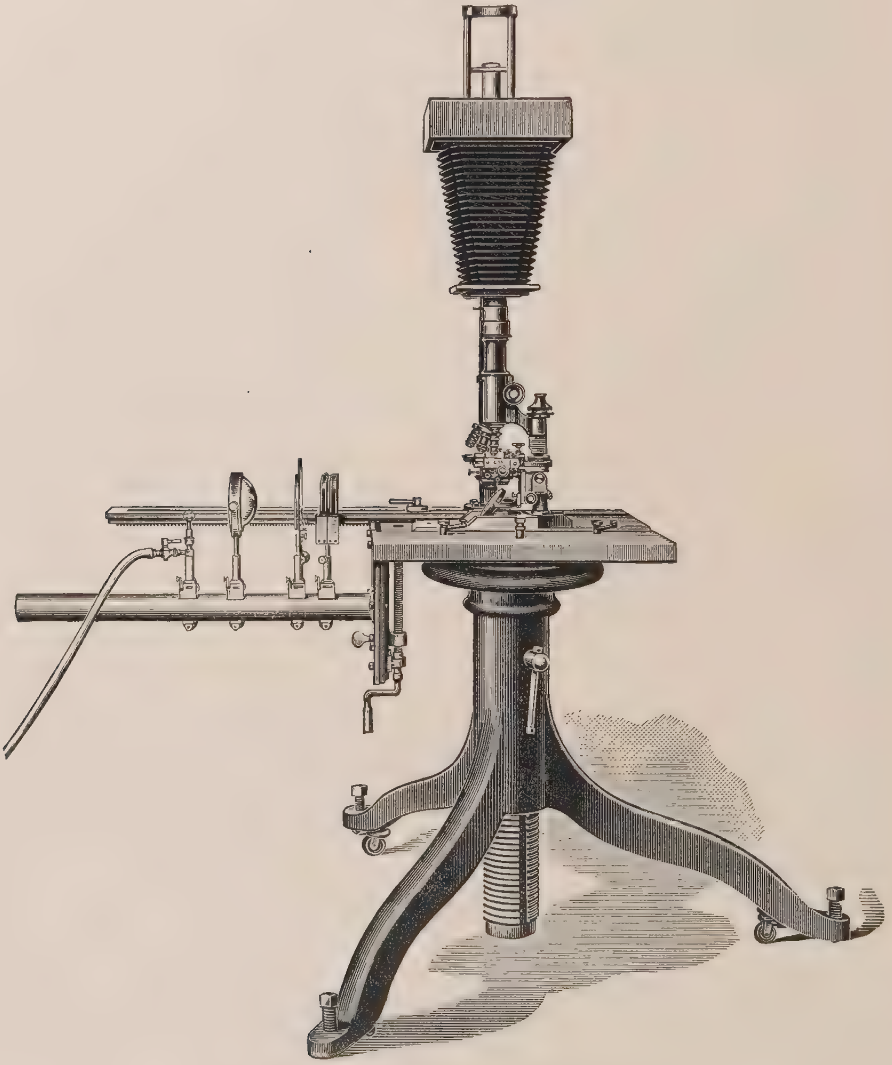


Arranged for illumination with arc light, camera and microscope at angle of 45°, table partly lowered.

more exact centering. This we have found to be an indispensable consideration, from the fact that no matter how carefully made, very slight differences in the manner in which the base of the optical part rests upon the optical bench, are sufficient to destroy the alignment, and it is a matter of great convenience to be able at will to adjust separately any piece of the apparatus both for height and transversely. The various portions of the illuminating apparatus may be removed from the tube forming the support, or be simply allowed (by removing the key which holds them upon the tube) to swing downward, and may be returned to their position where they resume their former place by means of the key falling into the slot in the tube.

The arrangement of the optical parts will be considered under Centering and Illumination. The microscope is fastened to the base plate by a transverse bar placed across the foot of the microscope and attached to the base plate by milled screws. When centered, a metal guide arranged on parallel arms is placed against the side of the microscope foot, and serves as a guide for the instrument in changing from the horizontal to the vertical position. The camera, as will be seen in Fig. 74, is supported upon the upright tube A, springing from the end of the plate D, which may be moved forward and backward by rack and pinion, the crank of which appears at C. The camera is supported upon two rails which are joined together at the ends and attached to the upright tube at their center by a pivot attached to a block through which the upright support passes. To the pivot upon which the camera is attached, is a segment of 90 degrees, slotted and fixed by a clamping screw B, so arranged that the camera may be clamped at any angle desired, thus making it possible to align the camera with the microscope at any position including and between the horizontal and vertical. The block through which the upright passes, and which supports the camera entire, is arranged with a clamping screw E, by means of which it may be clamped fast to the camera block. The supporting block is likewise arranged to clamp fast upon the upright, and has projecting from one of its sides a pin, against which strikes a pin attached to the camera block, thus furnishing a stop arrangement by which the camera support may always be returned to a fixed point. In manipulation, the screw of the camera block may be loosened and the camera swung around out of the way, merely resting upon the supporting block. It may be then returned to the position originally occupied, this being indicated by the contact of the pins projecting from the two supports. A second stop-pin projects from the outer side of the supporting block, and strikes upon a pin projecting from the base of the upright, which is so arranged that the alignment of the camera in the optical axis is obtained without further manipulation. Whether in the horizontal, inclined or vertical position the connection between the front of the camera and the microscope is made by a tube projecting from the front of the camera board into a larger tube which is placed over the tube of the microscope. Diagram B, Plate XXXVIII., shows a cross section of this arrangement. The sliding tube at 8, fastened to the front board, is so arranged that by a half revolution it slides forward a sufficient distance to make the connection, and when the camera is to be swung out of place the reverse movement withdraws the sliding tube from the larger tube on the microscope and the camera may then be swung clear. In the simpler apparatus, as manufactured by Winkel, the base plate of the entire apparatus is so arranged that it may be placed upon an ordinary wooden table and held fast by cleats which are screwed down over the projecting sides of the base plate. The complete apparatus is arranged with a massive iron foot, which to our minds greatly increases not only the solidity but the general utility of the apparatus, and we most heartily recommend this form of the apparatus as being by all means the most desirable, which, in spite of the increased expense, greatly increases the utility of the entire mechanism. This support consists of a heavy tripod through the feet of which are three adjustable screws. Under the feet of the tripod are placed three rollers, so arranged that the entire apparatus may be readily removed from one part of the room to any other without destroying the alignment of the apparatus. When ready to use, the screws are simply

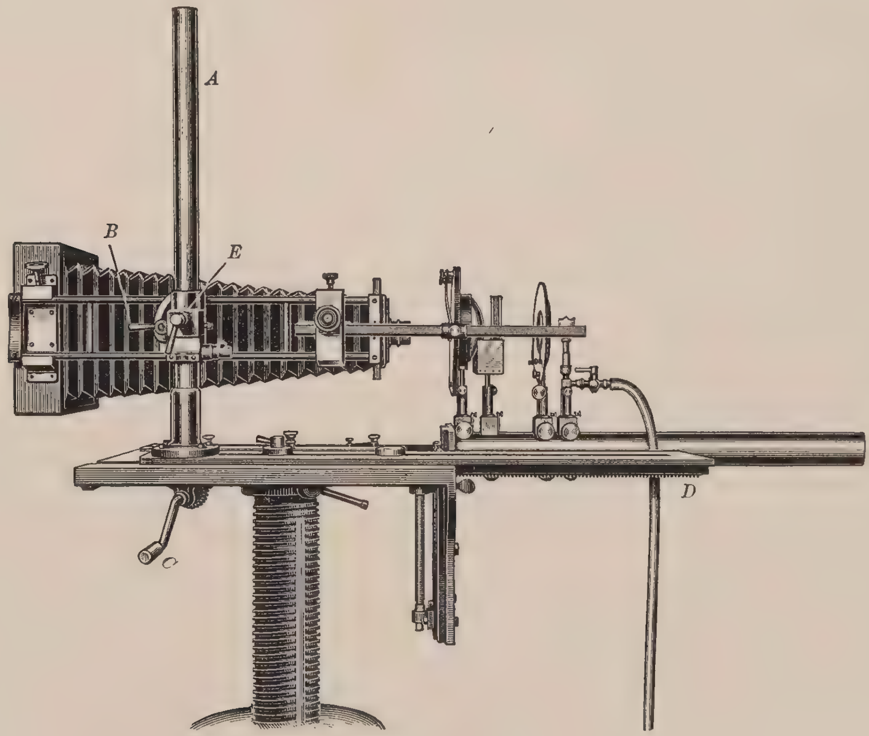
FIG. 73.



Vertical position, illuminating bench and table lowered.

screwed downward sufficiently to raise the apparatus off the rollers, and the greatest possible solidity is thus obtained, any inequalities of the floor being thus easily compensated. A distinct advantage in either form of this apparatus is that the operator can swing the camera aside, as indicated, and place himself squarely before the microscope. This could always be done with a large Zeiss apparatus, but as the space between the two tables was limited, it not infrequently happened that in taking or leaving the low stool provided with the apparatus, the operator came in contact with either part of the apparatus and thus disturbed the alignment. Beside this, in the Zeiss apparatus it was necessary for the operator to assume an uncomfortable position upon a low stool. In the apparatus being described the foot which supports the apparatus may be raised or lowered at will by means of a large vertical screw and a wheel placed upon the upper portion of the tripod. This arrangement is of distinct advantage where the apparatus is to be used by various operators, as the whole can be raised or lowered at will, so that the optical axis is brought directly opposite the eye of the operator. By long-continued operation this is a point of distinct importance, as the operator not infrequently found the unnatural position assumed in the employment of other apparatus produced considerable physical discomfort. The entire apparatus rests upon the vertical screw, and is so arranged that it can be revolved at will, which is a point of convenience in case it becomes necessary to employ any source of light not placed upon the illuminating bench, when by means of the ability to raise and lower the apparatus, as well as revolve it, the optical axis of the entire apparatus can be brought in line with a source of light placed at any convenient height upon a separate support. In employing the vertical position (Fig. 77) of the microscope the entire apparatus may be lowered to a height of 70 cm. from the floor, thus rendering it possible for the operator to look directly upon the ground glass of the instrument, instead of, as in the older Zeiss and other apparatus, being forced to mount upon some object of sufficient height before the image upon the screen of the camera could be observed. The entire apparatus having been once centered for itself, it may be moved at will from one point in the room to another,

Fig. 74.



Arranged for low power photography with micro-collinear objective, 90 mm. on front board.

or to an adjoining room; it may at will be centered to any source of light placed in a convenient position upon a separate support, or the whole apparatus may be placed before the window and sunlight or reflected light may be employed. The size of the camera is 13 x 18 cm. (5 x 7 in.) or a larger form of 18 x 18 cmm. is under consideration. A second and exceedingly useful possibility in the employment of the apparatus consists in the readiness with which low-power photographic lenses may be applied directly to the camera, the microscope being removed. The preparation to be photographed may be placed upon a special support or upon the face of the iris diaphragm, or a special stage, illustrated in Fig. 78, may be attached to the side of the camera, and by varying the distance of the parts of the optical bench photographs may be made without in any way altering the alignment of the apparatus, if it has been previously aligned in the horizontal position. In the hands of the authors this apparatus has been found to fulfill all possible requirements which can be placed upon a complete perfected apparatus. Its manipulation appears to be more complete than that of the larger apparatus in vogue, and it is in many ways much simpler than such apparatus.

RULES FOR ALIGNING THE PARTS OF THE PHOTOMICROGRAPHIC APPARATUS.

The parts are placed upon the illuminating bench in the following order: First, the support for the ray filters; second, the condensing lens with the convex surface toward the table; third, the iris diaphragm; fourth, the cooling trough, and fifth, the source of light. (Diagram, Plate XXXVIII.) The positions of 3 and 4 in special cases to be inverted. Before beginning the various steps of centering the apparatus the condenser lens and ray-filter support are turned downward.

The microscope in horizontal position without mirror, objective or ocular, but with the Abbe condenser in place, is placed upon the table of the photomicrographic apparatus, and the ends of the horseshoe foot braced firmly against the transverse strip directly in front of the illuminating bench. It is then lightly clamped in place by means of the screw and transverse block which press the microscope foot down on the table. The microscope must now be moved from side to side until its tube falls in alignment with the coupling tube of the front board of the camera. The parallel guide, used in changing the position of the microscope to the vertical, is now brought in contact with the side of the microscope foot. The microscope may be moved backward to any point on the table without destroying the alignment of the parts. The camera is now raised and lowered until the height of the sliding tube upon the front board is directly opposite the sleeve encircling the tube of the microscope. Care must be taken to see that the long axis of the camera is parallel to the sliding plate on which it is supported. Measure the distance from the surface of the table to the center of the condenser and adjust the parts upon the illuminating bench to the same height, beginning with the source of illumination, using for this purpose the mm. scale which is cut upon the supports. Center the diaphragm as nearly as possible, and so place it that a picture of the source of illumination is projected upon the closed diaphragm of the Abbe condenser of the microscope. This projected picture must fall as near the center of the diaphragm as possible. The iris diaphragm of the condenser is now opened. Two small caps are furnished with the apparatus, one of which screws into the screw at the lower end of the microscope tube and has a small hole 1 mm. in diameter at the center. A cap of similar construction fits over the ocular end of the microscope tube and has an opening at its center of about 2 mm. diameter. The last-mentioned diaphragm is now placed over the microscope tube, after which a small, bright circle of illumination appears upon the ground glass of the camera. This must be made to coincide with the center of the ground glass by slightly moving the microscope from right to left and raising and lowering the camera. When this is accomplished the supporting block under the camera support on the upright pillar on which the camera swings is screwed fast after the contact pins of these two parts have been brought in contact. By this means the camera may be swung aside, permitting direct observation through the microscope, and may be swung back into place, being stopped in position by the contact pins. Remove diaphragm from the ocular end of microscope tube and insert the small diaphragm in the objective end. A small circle of illumination will again be found upon the ground glass, and should fall at the center.

Replace the larger diaphragm over the ocular end of microscope tube, when both circles may be made out, one within the other. If these do not center upon the same axis and both fall at the center of the ground glass it indicates that the axis of the microscope and camera do not coincide. This is usually adjusted by slightly swinging the camera until it is perfectly parallel with the long axis of the microscope. Remove the condenser of the microscope, the field diaphragm and the ocular stop, when an inverted image of the source of light will be found upon the ground glass, and this must be centered by moving the source of light. The large collecting lens on the illuminating bench is now swung into place, when a large upright image of the source of illumination replaces the smaller inverted image upon the ground glass. This must be made to center by adjusting the collecting lens. Return the Abbe condenser, when a large inverted image of the source of illumination replaces the upright. This will usually be found to center if the previous steps have been correctly followed and the Abbe condenser is properly centered to the microscope stand. This last image will fall at the center of the ground glass. Where the microscope is supplied with an Abbe condenser arranged for centering by means of screws, the image does not usually fall at the center of the ground glass, and should now be centered by employing the arrangement attached to the condenser. Insert a low-power objective on the microscope tube, when a small inverted image will appear upon the center of the ground glass. Insert an ocular, when a large upright image of the source of illumination replaces the smaller image projected by the objective. If these steps have been properly followed out, the apparatus is now in perfect alignment. It is wise to repeat the various steps, noting that all the various images fall in succession upon the center of the ground glass. The intersection of two oblique lines drawn from the corners of the ground glass serve to indicate the center. The object to be photographed is now placed upon the microscope.

Koehler's Method for Equal Illumination of the Field.

All methods of illumination which employ a source of light having a limited area, may be divided into two general groups : those in which the image of the light source falls in the plane of the object, or in a plane adjacent to it, and those in which the image of the illuminant is projected through the object and falls at or near the point of exit of the cone of light. The great objection to the first class of methods consists in the great difficulty which is experienced in producing perfectly equal illumination of the visual field, especially where the surface to be illuminated is large, as in low-power photography. This difficulty has in the past been remedied by interposing a ground-glass screen between the source of light and the object, or by covering all but a small portion of the surface of the illuminant by the interposition of a diaphragm directly in front of the illuminant. But both of these expedients only partly accomplish the purpose for which they are employed and both greatly reduce the amount of available light. The interposition of a diaphragm in front of the illuminant has the further disadvantage that it prevents the use of two very admirable illuminants, the Welsbach burner and the limelight.

In a method devised by Koehler,[1] in which the image of the illuminant falls at a point near the exit of the rays of light from the instrument, we have at our disposal a method scientific in its foundation, certain and simple in application, and by means of which we may dispense with the interposition of ground-glass screens or reduction in the area of the source of light. Koehler's method employs as the source of light, not the illuminant itself, but an equally illuminated collective lens. The accompanying diagram (Plate XXXVIII., A) gives the course of rays from the source of light to the glass plate. It will be seen by tracing the rays of light in this diagram that not only is every point in the object lying at O′1, O′2 traversed by parallel rays of light, but that every point upon the ground glass between O‴1 and O‴2 is illuminated by rays of light coming from the entire surface of the source of light, L1, L2. The rays of light passing from L1, L2 intersect at the point O1, O2, at which point is introduced a diaphragm, the image of which appears in the plane of the object O′1, O′2. This diaphragm serves the purpose of reducing the extent of the object which is to be photographed.

At L′1, L′2 we have the iris diaphragm of the condenser, by means of which we may control the aperture of the cone of light passing through the condenser. At O′1, O′2 we observe the rays of light are parallel and that between the points O′1 and O′2 the illumination of the object must be equal. L″1, L″2 is the back focal point of the objective system, and at this point the rays of light again converge, forming an image of the source of light. At O″1, O″2 the rays of light again converge, producing the image of the object lying at O′1, O′2. At L‴1, L‴2 the rays again intersect, producing the final image of the source of light L′1, L′2, which, it will be observed, is at the point of convergence of the cone of light. After intersecting, these rays of light converge at O‴1, O‴2, producing upon the glass screen the image O″1, O′2, as well as the image of the diaphragm lying at O1, O2. The practical application of this method to photomicrography may be seen from diagram, Plate XXXVIII., B, in which the various parts upon the illuminating bench, the entire microscope system and the camera are shown in cross section. Koehler's method admits of great variation in the aperture of the cone of light and the extent of surface which is to be illuminated.

If the object is to be photographed with low power, and with a small diaphragm under the condenser, the collecting lens is placed at a distance from the condenser less than twice the focal length of the collecting lens.

If the object is to be photographed with high power and open iris the collecting lens is placed at a distance from the condenser greater than twice its focal length.

In the first case the image of the source of light projected upon the iris of the condenser will be small (Plate XXXVIII., B, 1′), the diameter of the equally illumined surface great (Plate XXXVIII., B, 2′); *i. e.*, the projected image of the field diaphragm, O1, O2, in the plane of the object.

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[1] Ein neues Beleuchtungsverfahren für Mikrophotographische Zwecke, Band X., Heft 4. Zeitschrift für Wissenschaftliche Mikroscopie.

In the second case the size of the image of the source of light will be large and will, therefore, cover the open iris of the condenser; the image of the field diagram small, however, sufficiently great to cover an area greater than the field of a high-power objective.

In the first case we have a large field of small aperture; in the second a small field of large aperture, in each case fulfilling the conditions required by the respective objectives.

Where the object is to be photographed by high power, the cone of light must have a greater aperture, but the extent of illuminated surface required is not so great. Therefore, the collective lens is placed at a point greater than twice its focal length.

To arrange the parts of the photomicrographic apparatus according to Koehler's method, we proceed as follows:

Having determined whether the preparation is to be photographed with high or low power, the parts of the apparatus are arranged in the following order, the preparation is placed upon the microscope stage and the point to be photographed is brought to the center. A low-power objective (40–60 mm.) is inserted in the microscope tube and focused upon the preparation. Looking through the instrument, we observe a faint image of the collective lens lying in the plane of the object. This must fall in the center of the field. The condenser is now racked to a point where the image of the collective lens in the plane of the object becomes sharp. This is best accomplished by placing a ground glass at the back of the collector and focusing upon the black ring in which the lens is mounted. When this is accomplished the ground glass is removed. The source of light is now moved back and forth until its image appears sharply defined upon the closed iris of the condenser. Viewed through the microscope, an equally illumined circular image of the collector appears in the field, against which the details of the preparation appear sharply defined. The field diaphragm (O1, O2) is now placed in front of or back of the collector, as the case may be, and moved to a point where its edges become sharply defined in the plane of the object. The low-power lens is now exchanged for the one to be used in making the photograph. The field diaphragm is now constricted so as to include just that portion of the field to be photographed. If these steps be properly followed, and the parts of the apparatus are in perfect alignment, the image projected upon the ground glass will be perfectly and evenly illuminated.

SOURCE OF LIGHT.

Petroleum Light.

This form of illumination need scarcely be considered to-day, when gas and electricity have been introduced into all institutes and dwelling houses. It has the disadvantage of deficient intensity, and requires proportionately longer exposure than the other sources of illumination. According to Neuhaus, properly arranged petroleum illumination requires but 13 times greater exposure than limelight.

Illuminating Gas

May be used by means of the ordinary burner, but this offers no advantage over the Welsbach mantle, which gives greater intensity of illumination, beside burning less gas for the same amount of illumination. This means of gas illumination has the distinct advantage that it is not influenced by currents of air, and gives a beautiful equally intense white light, beside presenting a large surface of equal illumination. An arrangement similar to the Welsbach mantle, but employing alcohol instead of illuminating gas, has of late been introduced, and should find employment in preference to the petroleum method of illumination. Fully one-half of the photographs in this atlas were made by means of a Welsbach burner with illuminating gas, and gave entirely satisfactory results even for photographs made with the oil immersion, the length of exposure never requiring longer than four or five minutes.

Acetylene Gas.

The introduction of acetylene gas has placed at the disposal of those who do not have at hand either illuminating gas or electricity, a form of illumination which has many desirable qualities, and which may be produced by means of inexpensive apparatus, many forms of which have been lately introduced in trade. The acetylene flame is of great intensity and beautiful white color. It presents an equally illuminated surface of sufficient size, and has but the one disadvantage that it is, as all free-burning flames, easily influenced by currents of air. The burners in vogue do not render it possible to burn large amounts of the gas, and its candle power is thereby limited. About one-third of the photographs in this atlas were made with acetylene gas.

Limelight.

Limelight has been largely superseded in practice by the Welsbach light and electric light, and owing to the inconvenience of manipulation as well as expense, may in the future be advantageously superseded by the acetylene illuminant.

Electric Light.

The best form of electric light is the arc light. The apparatus most in use in Germany is the arc light manufactured by Schuchart, which has the carbons at an angle of 45° to the plane of the condenser, thus giving a greater percentage of effective illumination than in the form in which the carbons occupy a vertical position. A still better form of arc light is the American arc light, manufactured by Thompson, in which the carbons occupy a position of 90° to each other, the negative carbon being placed in the optical axis. This arc light has the advantage of being simpler and of less weight than the Schuchart lamp, beside remaining centered and giving a great amount of available illumination. The hand-feed lamp has an attachment by means of which both the direct and interrupted current may be employed, which is a distinct advantage. This lamp is furnished in two forms, one with automatic regulation, and a more simple form for hand regulation. The automatic lamp is exceedingly convenient, and does not require as much adjustment as the hand-feed lamp, but where expense is a consideration the hand-feed lamp will be found in the hands of a careful operator to give exceedingly satisfactory results. The automatic form of this lamp adapted to the author's photographic apparatus, is shown in Fig. 72.

Preparation of the Specimen.

Preparations for photomicrographic reproduction should not, as a rule, be more than 4 to 6 mcrs. in thickness. In some cases preparations as thick as 10 or 12 may be used where the magnification is not too great. Paraffin sections, because they may be readily cut to the desired thinness, are best adapted for photomicrographic reproduction, but in select cases celloidin is to be preferred. Preparations which are to be photographed with low powers should be more deeply stained than those which are to be reproduced with a greater magnification. The best means of determining the proper amount of color is to examine the section in question with the objective with which it is to be photographed. The selection of a stain for sections which are to be photographed is not now such a matter of difficulty as previously, when the application of the principles of light were not so well understood as they are to-day. By the application of proper ray filters preparations stained with almost any combination of color may be faithfully and satisfactorily reproduced. One characteristic of a stain which is indispensable is that of transparency. Carmine possesses this quality to a high degree. On the whole, hæmatoxylin is the most useful of the nuclear stains and in most cases it is desirable to employ this stain alone, without the addition of a counter stain, such as eosin. Methylene-blue, owing to its lack of transparency, does not give as good results as hæmatoxylin.

RAY FILTERS.

The selection of a suitable ray filter for a preparation stained with any given color can best be determined by the application of the spectroscope. One of the various forms of pocket spectroscopes will suffice for this purpose. The most satisfactory instrument is a small spectroscope which is provided with a scale and a comparison prism. By means of the scale accurate readings may be made and with the comparison prism two colors may be readily compared. Such an instrument is shown in Fig. 76.[1] The construction of the instrument is shown in diagram, Fig. 75. With the eye placed at c (Fig. 75) the

FIG. 75.

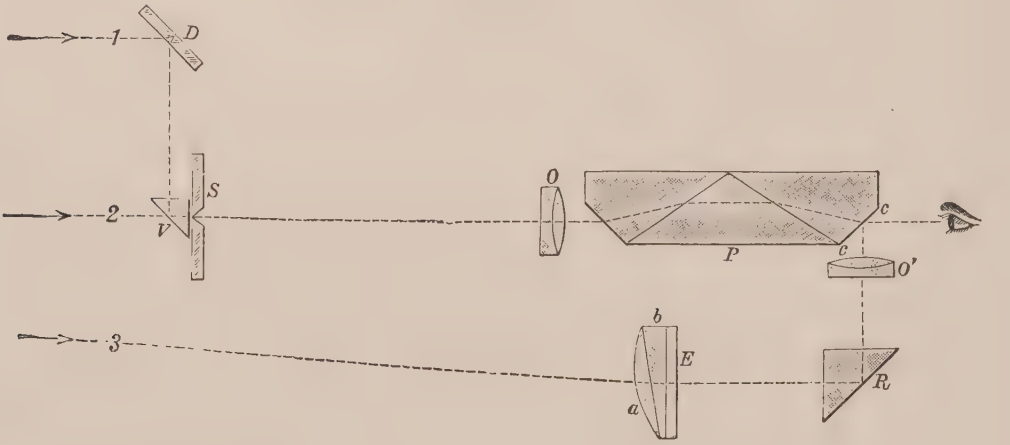
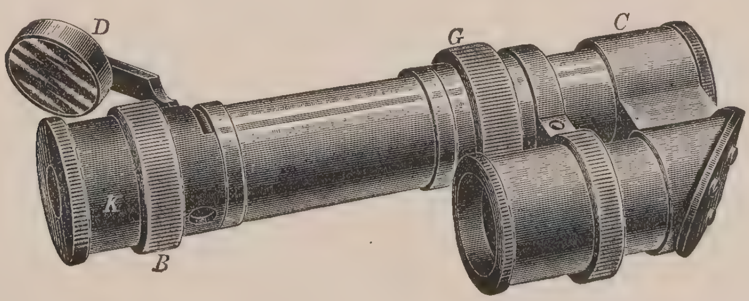


FIG. 76.



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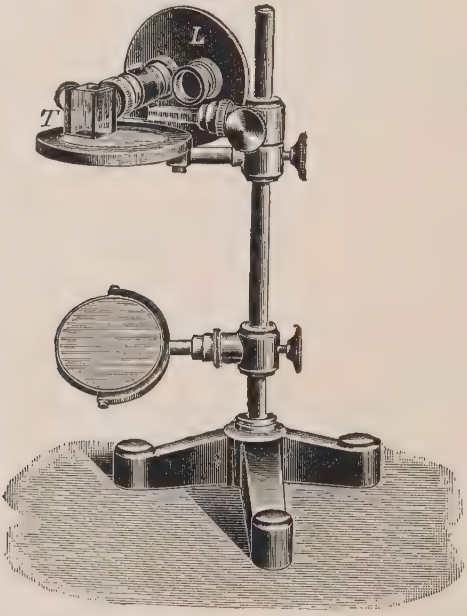
[1] The instrument described is made by Schmidt & Haensch, Berlin.

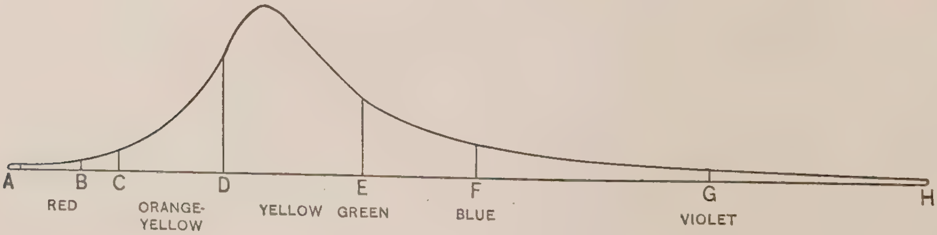
observer sees two superimposed spectra. One is illuminated by light taking the direction 1, the illumination for the other taking the direction 2. Over these is a brightly illuminated scale, the illumination for which takes the direction 3. For the investigation of absorption spectra, the trough containing the fluid can be placed over the opening at 2 when it may be compared with the spectrum of the source of illumination, which takes the direction 1. The width of the absorption bands may be determined by comparison with the numbers of the scale. The slit of the instrument is opened and closed by revolving the ring *B* (Fig. 76). The lens *O* is focused upon the slit by revolving the ring *G*. An exceedingly convenient attachment for this little instrument is found in a universal stand which is shown in Fig. 77. Upon this stand the instrument may be used either vertically or horizontally, as shown in the illustration. The trough *T* is placed before the opening 2 and a light shade *L* protects the observer's eye during the observation. A small instrument of this description, or even a simpler pocket spectroscope will fulfill all requirements in the preparation and control of ray filters.

Fig. 77.



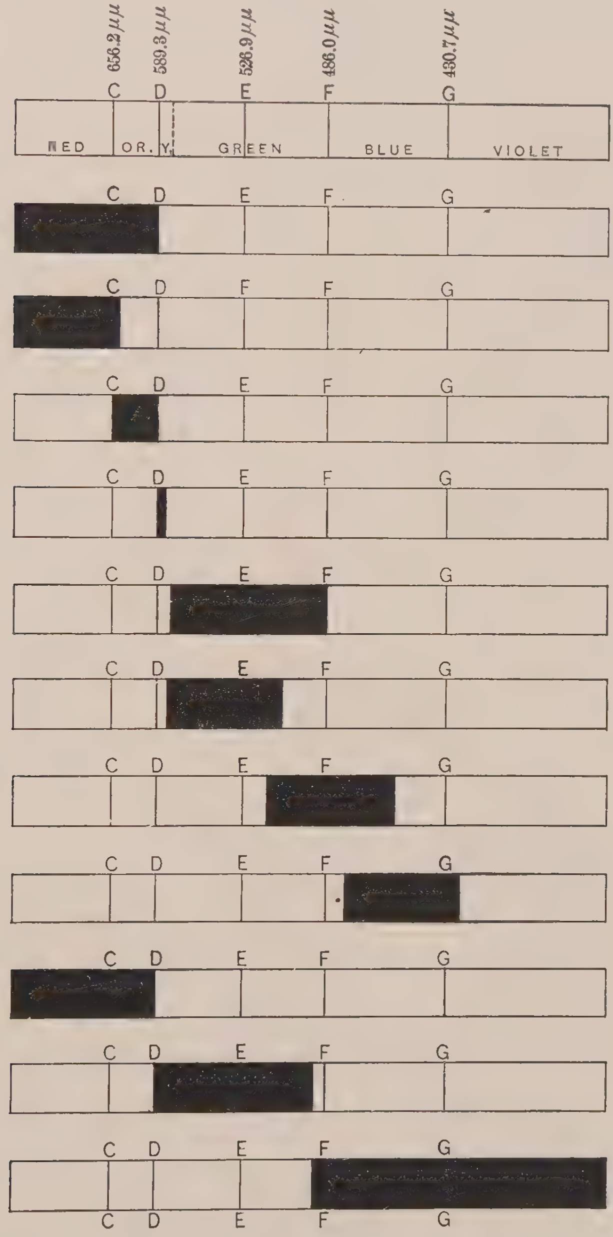
The principal consideration in making a photograph of an object which is to be printed in black and white, consists in reproducing the various colors in

Fig. 78.



the preparation in degrees of brilliancy, which shall correspond with the curve which represents the relative brilliancy of the various colors to our retina. When we observe an object which contains color, our retina receives two distinct impressions; one is that of the special physiological action brought about by the primary colors in the spectrum, and the other is an impression of relative brilliancy of the various colors thus observed, which intensity is expressed by the appended curve of Frauenhoff (Fig. 78). It will be noted upon

Fig. 79.



examination of this curve that the portions of the spectrum between C and F have much greater intensity than the colors lying at the ends of the spectrum, *i. e.*, between A and C and F and H. The maximum of this intensity lies between D and E in the yellow. It is, therefore, possible for us to reproduce a picture which originally contained colors, in degrees of black and white, which will produce upon the retina an impression of varying degrees of brilliancy, which may be made to correspond to this curve. If this be the case, the reproduction produces an appearance in which we recognize at once a great similarity between the colored original and the black and white reproduction. To accomplish this the red and blue violet colors in the original must be represented as much darker than the orange, yellow and green. It will thus be seen that in preparations containing red and blue, which is the usual combination, these two colors must be reduced to practically the same footing, that is, similar degrees of blackness if they are to present a normal appearance. If a preparation contain red or blue in combination with yellow, the yellow must be reproduced in a degree of gray which approaches white, whereas the red or blue would appear as a dark gray or black. The recognition of this principle has resulted in the greatest possible improvement in the reproduction of colored originals and has been rendered possible by the introduction of photographic plates, which are sensitive to various portions of the spectrum. To accomplish the reproduction of different colors in proportion to their brilliancy to the retina, it is necessary that we should have at our disposal a means of cutting out all but certain portions of the spectrum, as well as a photographic plate, which is sensitive to the portion of the spectrum which we employ. The following diagrammatic representation of various ray filters, taken from Popowitzky and Nägel (Fig. 79), includes a selection of filters which renders it possible for us to employ, separately, almost any portion of the spectrum. In the diagram the portions of the spectrum, which are indicated in black, represent the light which each ray filter transmits. These ray filters are to be employed in glass retainers and are to have a thickness of 10 mm.

No. 1 is massive ruby glass. (This ruby glass cannot be obtained in America, but may be purchased from any German dealer under the name of massive ruby glass.)

No. 2. Dilute lithium carmine solution. Proper amount of dilution should be determined with the spectroscope.

No. 3 is produced by the combination of two solutions placed in separate troughs, one in front of the other:

A. Mandarin yellow (*G* extra), 1 pt. sat. sol., 1 pt. water.
B. Copper sulphate (1 pt. sat. sol., 4 pts. water).

No. 4. *A.* Mandarin yellow (*G* extra), 1 pt. sat. sol., 3 pts. water.
B. Cupric chloride, sat. sol.

No. 5. Ammonia copper sulphate. (1 pt. sat. sol. cupric sulphate, 15 pts. water, 1 pt. ammonia 0.91.)

No. 6. *A.* No. 5 with
B. Bichromate of potash (1 pt. sat. sol., 10 pts. water).

No. 7. Methyl green and copper acetate, saturated aq. solution, to be diluted and mixed and tested with the spectroscope until proper degree of concentration is obtained.

No. 8. Potassium permanganate. (This solution likewise lets through a certain amount of red, and should be employed with ordinary plates which are not sensitive to the red rays.)

No. 9. Mandarin yellow (*G* extra), 1 pt. sat. sol., 1 pt. water.

No. 10. Cupric chloride, sat. sol.

No. 11. Ammonia cupric sulphate. (1 pt. sat. cupric sulphate, 6 pts. water, 1 pt. ammonia 0.91.)

It will be noted that Nos. 9, 10 and 11 exactly compensate for each other, and if the three mixtures of light transmitted separately by these ray filters were thrown upon the screen one over the other, they would give white light. To determine in each case which is a suitable ray filter to employ, it is only necessary to ascertain the spectrum of the color which is to be reproduced. If we desire the color shall appear as black, then the ray filter to be employed must transmit only light which is absorbed by this color. Having ascertained the absorption spectrum of the color to be photographed, any ray filter which transmits light which falls entirely within any of the absorption bands of the color to be photographed will cause the color to be reproduced as black. If we desire to have the color appear as white, then we must employ a ray filter which transmits exactly the same amount and quality of light as does the color itself. In other words, a ray filter made by a solution of the color to be photographed will cause the color in question to be reproduced as white.

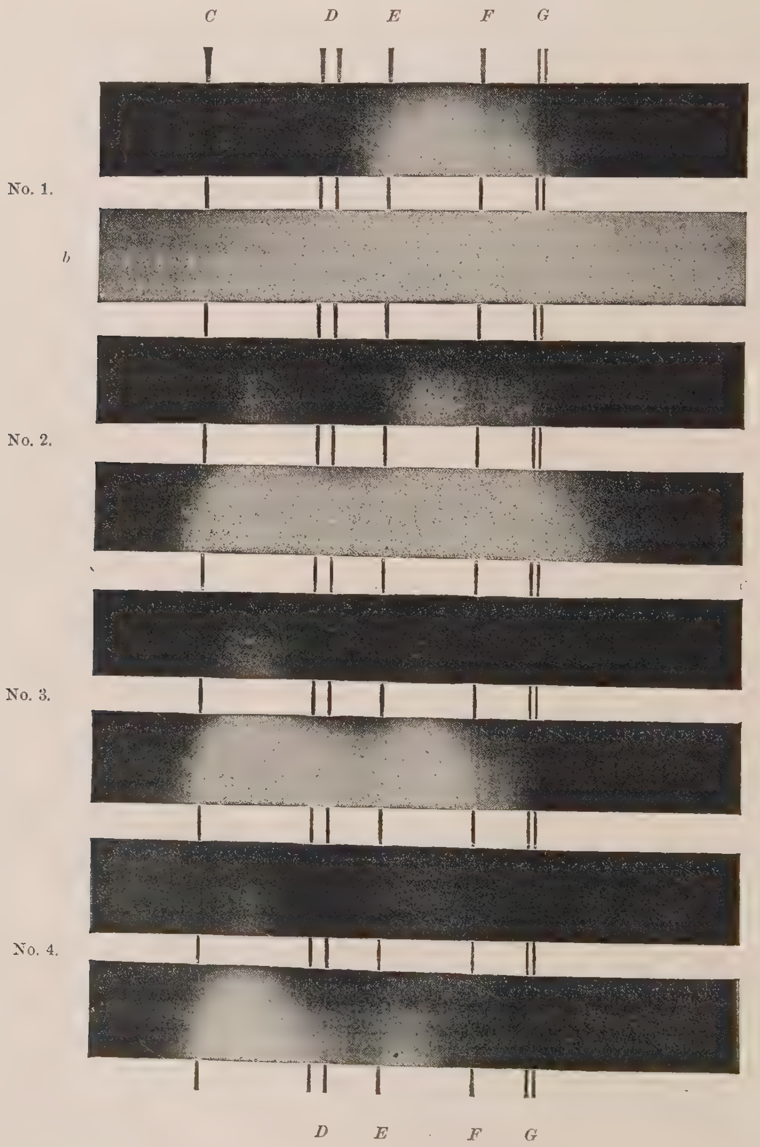
In reproduction in black and white the representation of the color in pure white is never desired, black or some shade of gray, with a proper degree of variation between the shades which represent the various colors entirely sufficing. For the reproduction of a preparation stained with hæmatoxylin in which we wish to have the stained portions appear black any of the ray filters from 1 to 6 will suffice. Owing to the difficulty of rendering plates sensitive to red light, we seldom employ a red ray filter for this purpose, as a yellow or green ray filter will produce exactly the same result, and plates which are sensitive to yellow and green are more easily prepared and manipulated. In photographing a preparation containing red and blue, a green ray filter will cause the red and blue to appear with relatively equal degrees of darkness, which produces the result desired. In cases where blue and yellow or blue and green occur together it is necessary to withhold the action of the blue rays in order that the yellow may appear with greater intensity. This is accomplished by introducing an orange screen, which does not entirely shut out the blue rays but reduces them in amount, thus enabling the yellow rays to produce a greater action on the plate during the time of exposure. This is illustrated in Fig. 80.

PHOTOGRAPHIC PLATES.

The old-fashioned wet plate and the original gelatine dry plate which is made by covering a plate of glass with an emulsion of a silver salt in gelatine and allowing the same to set or harden, were only sensitive to the blue rays of the spectrum, and until the discovery of Vogel of the action of pigments upon the photographic plate and the demonstration of his absorption theory of light, the blue rays of the spectrum were supposed to alone possess the ability to reduce the salts of silver, and were called the actinic rays of the spectrum. It has been found that the action of light in reducing a silver salt is brought about by the absorption by the silver salt of rays of blue light. Vogel found that if certain pigments, aniline dyes, etc., which absorb the light from other portions of the spectrum, were mixed with the emulsion the rays thus absorbed would likewise act upon the silver salt, and that the plate thus treated was rendered sensitive to the colored light absorbed. The character of the color of a pigment is due to the kind of light which it absorbs, the actual color being, however, produced by the light which it reflects. For instance, a red pigment absorbs the yellow and green rays of the spectrum, as well as the blue, and reflects the red either as pure red or a mixture in which red is the predominating color. This gives the pigment its color. Its action upon the silver salt would be indicated by the kind of light which it absorbed. Therefore, to render a photographic plate sensitive to yellow and green we would mix in the emulsion a red pigment.

The best orthochromatic dry plate is the eosin silver, made by Perutz in Munich, after the formula of Vogel and Obernetter. After a careful analysis of all the Continental and American brands of plates, the authors found that no other plate produces the same symmetrical spectrum which this plate possesses, and which may be so completely developed and manipulated without the production of fog.

FIG. 80.



The sensibility of the Perutz plate to the spectrum, as well as the action of light upon the ordinary dry plate may be seen in Fig. 80. These spectrographs were made with a large diffraction spectroscope, employing a plane Rowland grating.

No. 1, *a* and *b*, shows the sensibility of an ordinary dry plate. It will be observed that only that portion of the spectrum between *D* and *G* acts upon the plate. *a* is a short exposure; *b* a long exposure. In *b* the entire surface of the plate has been affected by reflection.

No. 2 shows short and long exposure of a Perutz plate. It will be observed in *a* that this plate is equally sensitive to orange and yellow rays of light between *C* and *D* and to blue between *E* and *G*.

No. 2, *b*, is a somewhat longer exposure, and shows the practical continuity of the spectrum between *G* and *C*.

No. 3 represents the action of the pale orange screen upon this plate. It will be observed in *a* that the blue rays of light are withheld and that a greater relative sensitiveness to the orange and yellow rays is thus produced. *b* shows the reduced action of the rays between *F* and *G* when the plate is exposed for a longer period of time.

No. 4 represents an exposure through a darker orange screen, and shows in *a* complete exclusion of the blue rays and in *b* a complete exclusion of the rays between *F* and *G*, and a marked reduction of rays between *E* and *F*. It will be seen from this diagram that the ordinary silver plate is only sensitive to a portion of the spectrum having a maximum between *E* and *F*, ceasing at *G* on one hand and extending slightly beyond *E* on the other. A plate sensitized with erythrocene, or more properly an eosin silver plate, gives practically a continuous spectrum between *G* and *C*. To render a plate sensitive to the red rays of the spectrum a blue pigment must be employed. The best pigments for this purpose are cyanine or nigrosin. An ordinary gelatine dry plate or eosin silver Perutz plate may be rendered sensitive to the red rays by bathing them in the following manner with solutions of these pigments.

Nigrosin.—The plate is immersed for two minutes for the purpose of softening the film and increasing the sensibility of the plate, in a solution prepared as follows: $\frac{1}{4}$ to 2 cc. ammonia, 0.96; distilled water, 80 cc.; alcohol, 95 per cent., 20 cc. It is then immersed for four minutes in a solution of nigrosin B solution (sol. 1–500) 10 cc.; aqua dest. 80 cc.; alcohol, 95 per cent., 20 cc.; ammonia, 0.96, 1 cc.

This manipulation is best performed in the dark, or if the plate to be sensitized is an ordinary gelatine plate the process may be carried out in monochromatic green light.[1]

Cyanine.—Cyanine must be employed in the form of chlorocyanine prepared after the formula of Eder.[2] The plate is immersed for two minutes in a dilute solution of ammonia prepared as follows:

Ammonia 0.96,	1–2 cc.
Aqua dest.,	80 "
Alcohol, 95-per-cent.,	20 "

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[1] For this purpose the author employs a lantern upon the face of which is attached an ordinary ray filter capsule filled with concentrated cupric chloride sol., to which is added a sufficient amount of picric acid to produce a narrow, green band. Such a lantern may be readily constructed by a tinsmith, and where electric light is available may be fitted with a small incandescent lamp. This lantern may likewise be employed in developing ordinary plates, and where massive ruby glass is not at hand the trough may be filled with lithium carmine solution.

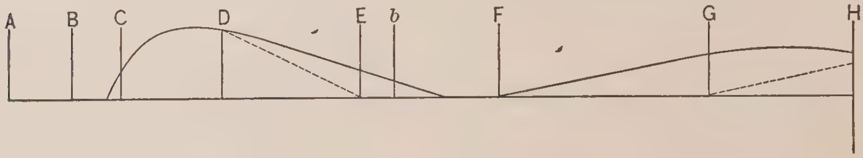
[2] Powdered cyanine is placed in a platinum dish, covered with water and hydrochloric acid evaporated over a water-bath with occasional stirring until dry. The residue is again moistened with dilute hydrochloric acid and again evaporated to dryness. During this last step hydriotic acid is driven off, leaving chlorocyanine, which, owing to a trace of hydrochloric acid does not appear perfectly blue. If the residue is carefully heated over a sand-bath or gauze net until the edges of the layer of cyanine become dark and metallic in appearance, this last remainder of hydrochloric acid will be driven off, but as ammonia is always added to the solution before bathing the plate any remaining trace of hydrochloric acid is neutralized.

It is then placed in a solution of chlorocyanine prepared as follows:

A freshly made 1–400 solution of chlorocyanine,	1–4 cc.
Alcohol, 95-per-cent.,	40 "
Aqua dest.,	80 "
Ammonia, 0.96,	1–2 "

Plates sensitized by either of these methods do not keep for more than a few hours, and are best exposed moist directly after bathing. If the plate is to be kept and dried it must be rinsed in distilled water and dried as quickly as possible. The sensibility curve of ordinary plates bathed in cyanine when exposed wet and dry is shown in Fig. 81 (solid line sensibility curve of plate exposed wet, dotted line curve of plate exposed after drying).

Fig. 81.



It will be seen that the plate exposed without drying possesses a greater sensibility to red than after drying. All plates rendered sensitive to the red rays require excessive care in manipulation and fog readily under forced development. It is, therefore, wiser not to develop the plate too long and to strengthen it with mercury afterward.

The Brothers Lumiere of Lyons have for some time placed upon the market a dry plate which has a considerable sensibility to red. For those who can obtain these plates they will be found more satisfactory than the bathed plate. Those who contemplate attempting three-color photography should learn the manipulation of collodion emulsion. Collodion emulsion is prepared by precipitating in collodion silver bromide or silver chloride. This emulsion may be prepared by the operator. Full instructions for the preparation and sensitizing of the emulsion may be found in the small works of von Huebel and Eder.[1] Collodion emulsion gives a beautiful, sharp negative in character very much like that obtained by the wet-plate process. The unsensitized emulsion is suitable for the production of positives and for the photography of colorless objects with blue or violet monochromatic light. The emulsion may be sensitized with any of the pigment sensitizers and when properly prepared and manipulated gives beautifully clear negatives. The great tendency to irregular distribution of the sensitizer as it is encountered in bathed dry plates is entirely overcome in this process, as the coloring matter is added to the fluid emulsion which is thoroughly shaken before pouring the plates. For three-color photography this emulsion and the necessary sensitizers may be obtained from Klimsch & Co., Frankfurt a. M. Any one, with a small amount of care, can learn to manipulate this emulsion and it will be found to give excellent results. The price of the emulsion, without duty, is about five dollars a liter. It keeps indefinitely, as the sensitizers are added just before use. One liter of the emulsion will suffice for the coating of a large number of negatives. Another excellent collodion emulsion is the Albert emulsion which may be obtained from Dr. E. Albert, Munich, or from Penrose & Co. in London. This emulsion is provided with an excellent orthochromatic sensitizer and for those who prefer, can be used in place of the Perutz plates.

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[1] Both of these works are published in German and are entitled "Die Collodium Emulsions Verfahren."

The Actual Steps in Making the Picture.

Having arranged the parts of the apparatus, the camera is swung into place and the connection between the microscope tube and the camera is made by revolving the sliding tube on the camera front board (Plate XXXVIII., Fig. B, 1) into the encircling tube, 2. The ground glass is now placed upon the camera and the bellows are moved forward and backward until the desired diameter is obtained. The ray filter to be used in making the photograph is now inserted at a point between the collector and the condenser. The image is then roughly focused and the ground glass is exchanged for a plate of plane glass, upon which, by means of a magnifier the image may be observed. Considerable dexterity is required in focusing the image sharply upon the plain glass screen. First of all, the operator must determine that the magnifying glass is exactly focused upon the front surface of the screen. To accomplish this a few ink marks may be made upon the front surface of the glass screen upon which the magnifier is to be focused.

Having determined this point, the operator begins at the center with the magnifying glass held against the glass screen, and with his left hand reaches over and controls the micrometer screw of the microscope. He now focuses the image at the center sharply upon the screen, and gradually moves the magnifier towards the periphery, repeating this operation until he has determined the position in which the definition at the periphery and in the center produces the best average effect.

The operator must determine the amount of illumination necessary to produce the best definition and contrast in the picture. This is accomplished by observing the image and gradually closing the iris diaphragm beneath the condenser.

Having focused the image and having determined the proper amount of illumination, the plain glass is removed and the plate-holder containing a plate sensitive to the rays of light which are transmitted by the ray filter takes its place.

The operator now places a black screen or interposes his hand directly in front of the condenser while the plate holder is being opened. After the plate holder is opened the operator allows a few seconds for vibration to cease and then makes the exposure.

No definite rule for the length of exposure may be given. The time which a plate should be exposed depends upon the amount of illumination, the character of the image and the sensitiveness of the plate. The best means for determining the length of exposure is to study the brilliancy of the image on the screen, as finally focused, for this purpose employing the ground glass. By this means the operator can form a general idea as to the length of exposure necessary for a given plate.

DEVELOPING THE PLATE.

If the plate is sensitive to blue, yellow or green, it must be developed in ruby light obtained either by a lithium carmine filter or by massive ruby glass. If the plate be an ordinary bromide silver gelatine plate sensitized with nigrosin or cyanine it is best developed in the light of a green ray filter.

Developers.

For photomicrographs a developer giving a clear sharp negative is the most desirable. Sufficient density is likewise a necessity. It will be found that different developers act best with different brands of plates.

For the Perutz plate Rodinol 1–18, with plenty of potassium bromide 10-per-cent. solution added, gives a good negative. Ortol (A. G. für A. F., Berlin) is equally good. The best formulæ are the following. The solutions are prepared separately and are lettered A and B.

Ortol.

A.

Water, cold,	1,000 cc.
Potassium metabisulphite,	7.5 grms.
Ortol,	15 "

B.

Water,	1,000 cc.
Soda carbonate crystals,	120 grms.
Sodium sulphite,	180 "
KBr,	1–2 "
Sodium hyposulphite sol.,	10 cc.

Ortol Potash,

B2.

Water, cold,	1,000 cc.
Potash,	60 grms.
Sodium sulphite crystals,	180 "
KBr,	1–2 "
Sodium hyposulphite,	10 cc.

(For Ortol soda A and B are used; for Ortol potash A and B2.)

For quick and hard development equal parts of A and B; for soft, slow development add an equal part of water. For plates sensitized with cyanine or nigrosin add to every 100 cc. of developer 8–10 drops of 10-per-cent. solution of neutral potassic chromate (K_2CrO_4). This last is added to prevent fog.

Glycin (A. G. für A. F., Berlin).—This is a most excellent developer, and produces an exceedingly clear, sharp negative—

Aqua dest.,	1,000 cc.
Dry basic sodium phosphate,	125–200 grms.
Glycin,	15 "
Sodium sulphate, free from water,	40 "

The solution is used as prepared. This developer is especially adapted for lantern slides.

Pyrogallic Acid.—Those who have worked with pyrogallic-acid developer will find this a suitable developer, but it has the disadvantage of staining the hands, and must be used directly after mixing.

A reliable formula is the following:—

A.

100 cc. aqua dest., 2 gr. potassium metabisulphide, 10 gr. pyrogallic acid.	This solution will keep in well-stoppered bottle for one year.

B.

100 cc. aqua dest.,
10 gr. soda crystals,
20 gr. neutral sodium trisulphite.

Hydrochinon does not produce quite as clear negatives as glycin and pyro, but has greater covering power, thus producing a denser negative. An excellent formula for hydrochinon developer in one solution is the following:

Aqua dest.,	1,000 ccm.
Sodium sulphite,	40 grms.
Hydrochinon,	5 "
Potassium carbonate,	51–75 "

The sodium sulphite is dissolved first, then the hydrochinon, and when all is dissolved the potassium carbonate.[1]

As a rule orthochromatic plates will not stand prolonged development without fogging. To prevent this, add plenty of potassium bromide, sol. 10 per cent. Do not expose the plate unnecessarily to either the red or green light. If the plate be large it should be rinsed in water before placing in the developing tray, and the developer should be poured over the plate in one sweep. A cover of suitable size should now be placed over the developing tray, and the tray rocked gently from side to side during development. The plate should be examined from time to time to observe the progress of development, and when the shadows have disappeared the back of the plate should be observed as quickly as possible to prevent unnecessary exposure to the light in which the plate is developed. When the image has come through on the back and the shadows have again disappeared the development should be broken off. The plate is now rinsed in water and fixed in an acid fixing bath, of the following composition:

A.

Sodium hyposulphite,	500 grms.
Water,	1,000 cc.

B.

Sodium sulphite,	250 grms.
Water,	1,000 cc.

HCl 70 cc. or 30 cc. concentrated H_2SO_4.
Mix 1,000 cc. A and 80 cc. B.

This fixing bath has the advantage of working rapidly, producing a clear negative and preventing staining of the negative.

Perutz plates, and in fact all plates sensitized for other portions of the spectrum than blue, must be thoroughly fixed out before exposure to the light. If this precaution be not taken the plates will occasionally show a yellow fog, which cannot be removed by any known means.

After fixing, the negative must be washed for several hours in running water, after which, if it does not show sufficient density, it may be strengthened by the following method:

Corrosive sublimate,	3 pts.
Glacial acetic acid,	1 pt.
Water,	100 pts.

---

[1] Further formulæ and details regarding development can be obtained from the many good text-books on photography. An excellent book of this sort is that of Schmidt, Compendium der practischen Photographie.

This solution should be kept in a large-mouthed, well-stoppered bottle, and may be used repeatedly. The plate is now immersed in this solution, whereupon, the black silver picture takes on a white or grayish color, with increased intensity produced by a white or grayish deposit (Ag_2Cl, Hg_2Cl_2). The plate is now thoroughly washed. The deposit of mercuric and argentous chloride upon the plate, owing to its gray or whitish color, produces no noticeable strengthening of the negative when printed in this condition. It is, therefore, desirable to change the deposit into one of darker color, and for this purpose various subagents may be employed, the best of which are ammonia and sodium sulphite. These solutions reduce the mercurous and argentous chloride into the metal, which is of a dark brown or black color. To accomplish this the plate is first thoroughly washed and placed in a dish of distilled water to which has been added a few drops of ammonia or a 10-per-cent. solution of sodium sulphite in distilled water. The plate must now be thoroughly washed, after which it is put in a suitable rack in a locality free from dust, to dry.

If the plate has been over-developed and is too dense it may be reduced by the following method :

Ferrocyanide of potash, 10-per-cent. sol.,	10 cc.
Hyposulphite of soda, 12-per-cent. sol.,	100 cc.

These two solutions are mixed before using, and the plate is immersed in the solution. The plate must be removed often and rinsed in water and examined to determine the progress of reduction. When it has been reduced to the proper density it must be thoroughly washed, after which it may be dried.

POSITIVE PROCESS.

The impression which is produced by light acting through a lens upon the gelatine silver film of the ordinary dry plate, forms what is known as a negative. The image in a negative is the opposite, as to light and shade, of the original. This is easily understood when we remember that the light acting upon the plate reduces the silver, which, under the action of the developer, appears in the form of black granules, and where the light does not act the silver is not reduced and in the fixing process is removed. Therefore, in order to reproduce the original as it appeared we must make from the negative a second impression called a positive. The positive is made by exposing through a negative either a sensitized paper or a sensitized glass plate. In the first place we produce what is known as a silver print; in the second a glass transparency, or a diapositive. There are various forms of printing paper, all of which are more or less suited to the purpose, and as these papers are toned and fixed according to special formulæ, it is not necessary here to go into the details of their manipulation.

There are a few rules in printing which it is desirable to observe. A photomicrograph usually requires contrast; therefore, a paper producing prints which possess contrast is the most desirable. It is always well to make a sufficiently dark print before toning, and care should be taken that the prints are carefully washed after fixing.

LANTERN SLIDES.

Lantern slides are made either by exposing a suitable plate under a negative or by placing the negative in a lantern slide camera and photographing it upon a lantern slide plate. This last step is necessary where the size of the negative does not conform to the requirements of the projection apparatus. The best lantern slides are made by the wet-plate process or with collodion emulsion. For those who do not understand the manipulation of wet plates or collodion and have not the necessary equipment excellent results may be obtained with various brands of so-called lantern slide plates. Among the best of these are the Eastman lantern slide plate and the transparency plate of Forbes, Rochester. The manipulation of the diapositive for lantern slides is exactly the same as that of ordinary negatives. Special developing formulæ accompany these brands of plates, and in obtaining color effects the steps recommended by the makers of the plates should be observed. When the lantern slide positive is prepared it is covered by a suitable mat and cover-glass to protect the film, the edges are bound with gummed paper and the slides may be filed away in a suitable cabinet or box.

TRI-COLOR PHOTOGRAPHY.

The idea of photographing the component colors in an image, each color upon a separate plate, and then by a mechanical process printing these colored images in their appropriate colors, one upon the other, originated shortly after the discovery by Vogel of the orthochromatic plate. It is not necessary to photograph every color in the original in order to reproduce it. Three colors equidistantly placed in the spectrum when printed together will give, by mixing, the intermediate colors. For this purpose red, yellow and blue have usually been employed. The greatest difficulty in the process consists in the impurity of printers' inks. A printers' ink consists of a finely granulated opaque coloring matter fixed upon a basic substance mixed in a suitable fluid usually a varnish which serves the purpose of holding it together and insuring the spreading of the finely powdered coloring matter upon the paper. Examined spectroscopically, almost all the printers' inks in use will be found to consist either of colors which are not true colors or of mixtures. According to Von Huebel a true color is one which has its principal absorption band in the complementary color; and practically none of the printers' inks in use fulfill these conditions. Another defect in the process, and one which cannot be remedied, is that the picture printed in pigment must of necessity be a few shades darker than the original. This is due to the fact that if we mix the lights which compose the spectrum we have white light; whereas, if we mix the same in pigments we produce black. Therefore, as the various colors printed upon each other mix they approach toward black, and, the entire picture is darker than the original. These are of course limitations, but the introduction of color in the picture is in some cases so important that we may entirely disregard these minor defects.

In order to separate the red, yellow and blue in the original, it is necessary to employ for each a ray filter which shall absorb the color to be reproduced. The first step in adjusting the ray filters is to pin up a chart upon which are printed in equal intensity, the three printers' inks in which the reproduction is to be printed. These must be illuminated, with the source of illumination which is to be employed in making the photograph. A solution of the different inks is now made in benzol and these are examined with the spectroscope for the purpose of obtaining a rough idea as to what light they reflect. The colors should be so distributed in the spectrum that none of them possess common absorption bands or overlap each other. Having obtained the rough absorption spectrum of each color a ray filter must be selected which will absorb one of the colors and permit the other two colors to pass. When the ray filter is prepared the printed colors should be viewed through it by the observer, when the color which is to be reproduced should appear black, the other two retaining their original color. A plate properly sensitized for the two colors which are to be photographed should be employed. If the plate is not equally sensitive to the two active colors this may be corrected in collodion emulsion plates by varying the amount of sensitizer which is added to the emulsion. Having properly adjusted the ray filters to the colors and having demonstrated the correctness of the adjustment by making test negatives, the object to be photographed is first focused through that ray filter which transmits light nearest the center of the spectrum, after which a negative is made through this ray filter upon a properly sensitized plate. Without altering the arrangement of the parts of the apparatus, the ray filters are changed and the remaining negatives are made, correcting the focus at each exposure. With apochromatic objectives the three images thus photographed will be found to be equally sharp. With an achromatic objective this is not the case, as will be seen from the diagram (Plate XXXVIII., C). With low power objectives, even the ordinary achromatic objective, the focal difference is so small as to make no serious difference ; with the micro-collinear and planar objectives there is no focal difference, all three images being produced with equal definition. The three negatives may be reproduced by heliotype, or half-tone, which is in most cases more desirable, as greater accuracy in registration can be obtained by this method. Positives must first be made, and from these the necessary half-tone negatives are produced by the usual methods. In printing the colors are applied to the paper in the order of their brilliancy, yellow first, then red, and blue last. Von Hübl has recently shown (Archiv f. wissent. Photographie) that red, yellow and blue are not the colors best suited for three-color reproduction, but that yellow, blue-green, and purple, when suitable inks are found, will give us absolutely accurate results. At present this improvement has not been introduced into commercial three-color reproduction, but we may confidently expect that in the near future the process may reach practical perfection.

REPRODUCTION PROCESSES.

Various methods for the reproduction of photographic positives, so-called photo-mechanical processes, are in use. Certain of these processes possess the ability to reproduce from the negative without too great loss of definition. Others need not be considered, because of the great loss in definition which results from their employment. Those which may be considered in connection with photomicrographs are : First, the heliotype (gelatine process, or Lichtdruck). This process depends upon the reaction which occurs in gelatine sensitized with chrome salts after exposure to light. The thus sensitized gelatine coating is so altered by exposure through a negative that the portions which are acted upon by light become insoluble and likewise lose their ability to absorb water ; whereas, those positions of the plate not acted upon by light will absorb water. If such a plate be inked with grease ink the portions of the plate which have taken on the water, *i. e.*, the portions not acted upon by the light, the high light in the positive, will not take on the ink, and the portions which have been acted upon by the light take the ink, and a print thus made will give us a positive picture which may be printed upon paper. A gelatine plate so made is capable of giving a certain number of impressions, dependent upon the character of the subject and upon the amount of moisture which the plate contains. The process is, therefore, influenced by the conditions of the atmosphere, and requires considerable skill to produce a large edition of prints of uniform quality. The heliotype plates in this book, by the Heliotype Co., Boston, have been made by this process. This process produces the original with a minimum loss in definition, and owing to the difference in cost between it and the photogravure, should be placed first.

Photogravure.

Various forms of this process are employed, but in principle it consists in etching upon a copper plate through a negative chrome gelatine film. The chrome gelatine layer, when exposed to light, becomes insoluble and by a process of washing the soluble portions of the gelatine are dissolved. If in the chrome gelatine emulsion a pigment be mixed a picture will result, produced by the relative thickness and thinness of the remaining film. Such a film offers a certain resistance to etching fluids, that is, the etching fluids will act more quickly through the thin parts of the film than the thick. If such a film be transferred to a copper plate and an etching solution, usually chloride of iron, be poured upon the film, the etching fluid will pass through the thin portions of the film more quickly than the thick portions and produce upon the plate an irregularity of surface, corresponding to the high lights, half tones and shadows of the gelatine film. It will be seen that the gelatine film must be a negative, and to prepare this a glass positive must be made from the original negative and through this must be exposed the gelatine film. The picture which is etched upon the copper plate is a depressed picture, therefore in printing the ink would not be readily taken up by the paper from the deep portions and in order to give the shadows a similar elevation to the high lights, it is necessary to protect the surface of the copper plate in these portions before etching.

To accomplish this the plate is dusted with powdered asphaltum and heated. As a result, the fine particles of asphaltum turn into minute droplets attached to the plate, and which thus protect it from the etching fluid. If the plate be now etched the etching fluid can only act upon the plate between the droplets of asphaltum. If the action of the etching be but for a short time the protection of the surface by the drops of asphalt will be such that the intermediate spaces are eaten away by the etching fluid directly downward from the edges of the drops. If the action continue somewhat longer it will begin to undermine the drops, and when the asphalt is removed we find the elevations which represent the drops either of the same size as the drops of asphalt or undermined and smaller. By this means, where the action of the etching fluid has been short the metal elevations will lie close together and will not take on as great an amount of ink as the portions of the plate which have been eaten away beneath the drops by the prolonged action of the corrosive. By this means where the gelatine layer is thick and action of the gelatine fluid short the unetched surface will be great and the amount of ink held by the plate will be small, and we have a high light. Where the action is prolonged a greater surface of the plate is etched away, and we have a shadow. These plates must be printed with great care upon a hand press, and the cost of reproduction by this method is, therefore, high. Owing to the grain which is introduced by the application of asphalt, the definition of plates reproduced by this method is not as good as by heliotype. The process possesses the advantage over the heliotype method that a large edition of prints may be made from a photogravure plate without apparent diminution in quality.

Half-Tone Process.

The half-tone process consists in introducing into the copper plate a grain similar to that required in photogravure, but the means by which this grain is produced is entirely different. In half-tone the necessary grain is obtained by optical means.

A glass plate, or rather two glass plates, cemented together with balsam, upon which fine lines have been ruled, etched and filled with pigment, the lines on one plate being placed at an angle of 90 degrees to the lines upon the other plate so as to produce a fine network, is interposed between a photographic objective and a sensitive plate in a camera. If a photograph be made upon a plate through such a grating there will appear upon the plate first the image of the object projected upon the plate by the photographic lens, and second from the grating a series of pinhole images of the illuminated diaphragm in the objective. For each opening in the grating the light which passes through it and acts as the illuminant of the diaphragm in the objective must emanate from a certain portion of the object. Therefore, we have a series of dots projected upon the plate by the interspaces in the grating; those produced by rays of light from the shadows of the object being smaller and less intense than those produced by the light emanated from the brightly illuminated portions of the object. If the diaphragm of the lens be round the projected images of the diaphragm will be round, and to alter the character of the dots projected upon the plate various forms of diaphragms are employed. A negative thus produced contains a picture of the object produced by minute dots small in the shadows and larger and more intense in the high lights.

This is now printed upon a plate coated with chrome gelatine emulsion, and after proper exposure the copper plate is etched much after the manner of photogravure, with a suitable etching fluid. A plate so etched will take ordinary printers' ink, and if the grain be coarse enough, may be printed upon an ordinary press. The principal drawback to this method for the reproduction of microphotographs is that the coarse grain destroys all of the finer details. The extent to which the details are obliterated depends upon the fineness of the dots and their distance from each other, and this again is dependent upon the fineness of the grating employed. For ordinary commercial purposes about 100–150 lines to the inch are employed. The half-tone reproductions of apparatus in this book are made with a screen of 165 lines to the inch. For three-color reproductions of photomicrographs a 200-line screen for the coarser subjects, a 300-line screen for the finer subjects will suffice. Recently Levy, in Philadelphia, has succeeded in producing screens of 400 lines to the inch. The fineness of the dots produced by this screen is such that the eye cannot differentiate between them, and in half-tone reproductions made by this screen the grain of the picture is practically obliterated (see Pl. XXVII.). For ordinary photomicrographs the 400-line screen will in part replace heliotype. A peculiarity of this method is the almost total obliteration of the middle tones of the picture, the whole appearing harder than in the negative. Therefore, soft negatives are essential where this process is to be employed.

In reproducing photomicrographs in half-tone or three-color negatives in half-tone, it is necessary first to make positives from the negatives and from these half-tone negatives are made through a grating and these are printed upon the copper plates. Half-tone has the great advantage that the plates may be printed upon an ordinary press and a large number of prints may be made from one cliché. For three-color reproduction, half-tone blocks made with a fine grating are preferable to heliotype, owing to the difficulty of obtaining perfect registration with the latter method. In order that the three colors may be printed over each other, it is necessary that each color should be allowed to dry, and this is accomplished by printing on three successive days, which, likewise, introduces a difficulty where heliotype is concerned.

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